PRECLINICAL SAFETY EVALUATION OF

RATHI NAGARA RASA MEZHUGU

The dissertation Submitted by

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled “Preclinical safety evaluation of **Rathi Nagara Rasa Mezhugu”** is a bonafide and genuine research work carried out by me under the guidance of Dr. R. Madhavan, M.D(S).Department of Nanju Noolum Maruthuva Needhi Noolum, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

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BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr.M.Veerasivaraman**, (**Reg. No: 321516207**) a student of final year M.D(s), Branch-VI, **Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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1. INTRODUCTION

"சொல்லிடவே தேவிக்குச் சதாசிவன்றான்
சொல்லவே தேவியும் நந்திக்கு சொல்ல
நல்லிடவே நந்திதன் வந்திரிக்கு சொல்ல
நயமுடன் தன்வந்திரி யசுவினிக்குச் சொல்ல
அல்லிடவே யசுவினியாத் தேவர் தாமும்
அக்த்தியர்க் குரைத்திடவே யம்மு னீந்திரன்
புல்லிடவே புலத்தியர்க் குபதே சிக்க
புலத்தியரும் தேரையர்க்குப் புகன்றிட்டாரே"

– யூகி வைத்திய சிந்தாமணி 800[1]

Siddha system of medicine is the most ancient medical science which is propounded and practiced by eminent spiritual scientists called Siddhars. Siddhars are those who lived and maintaining their bodies as they desire best. Siddhars know the importance of a healthy body. They were ever young and healthy.They analysed the importance drugs for a strong physique and had wonderful medicine with them to keep the body imperishable. Thus they attained the eight Siddhis. (Attama Siddhi).

Both an ascetic and a common man are used to take care of his health was said by the Siddhar Thirumoolar:

"உடம்பார் அழியின் உயிரார் அழிவர்
திடம்பட மெய்ஞானம் சேரவும் மாட்டார்
உடம்பை வளர்க்கும் உபாயம் அறிந்தேன்
உடம்பை வளர்த்தேன் உயிர் வளர்த்தேனே"

– திருமந்திரம்[2]

In Siddha system of medicine, there is a close relationship between man and universe (Prabancham). Whatever changes occur in the prabancham, influences the human body also. It has been illustrated as,

"அண்டத்தில் உள்ளதே பிண்டம்

பிண்டத்தில் உள்ளதே அண்டம்

அண்டமும் பிண்டமும் ஒன்றே

அறிந்துதான் பார்க்கும் போதே"

– சட்டமுனி ஞானம்[3]

Siddha system of medicine practiced in South India has number of remedies for various ailments as mentioned in Siddha pharmacopoeia which include drugs of

- Mooligai (Herbal)
- Thathu (Inorganic substances)
- Jeevam (Animal products)

According to their mode application the Siddha medicine could be categorized into two classes:

1. Internal medicine
2. External medicine

Among 32 internal medicine mezhugu is one among them and its shelf life is for 5 years.

Mezhugu :

This is two types:

a) Araippu mezhugu (obtained by grinding drugs)
b) Churrukku mezhugu (obtained by heating them by adding oily substances)[4].

Toxicology is a science of poisons which includes detection and knowledge about the nature and effects of poisons as well as treatment of poisoning[5]. The goal of toxicity assessment is to identify adverse effects of a substance. The relationship between dose and its effects on the exposed organism is of high significance in toxicology. Siddhars are well known about the toxic effect of drugs and their specific antidotes. In Siddha system there are so many medicines which are used for various diseases. Some medicines are prepared using metallic and mineral compounds after their purification.

Nowadays there is a need to evaluate the toxicity of such drug formulations. One of the formulations ***Rathi nagara rasa mezhugu*** mentioned in anuboga vaidhaya naveneetham[6]. The ingredients of Rathi nagara rasa mezhugu are metallic and herbal compounds like Rasam (Mercury), Ganthagam (Sulphur), Serankottai (*semecarpus anacardium*) cures the disease of Lingaputtru (Penial cancer), Algulputtru (Cervix cancer), Araiyaappu (Adenitis), Kandamaalai (Cervical adenitis), Karunkuttam, Senkuttam (Leprosy), Megaranam (Syphilis), Kaalkai mudakku (Rheumatoid Arthritis).

So far there is no scientific rationale carried out for its toxicity profile, hence I proposed to take Rathi nagara rasa mezhugu for my dissertation study to evaluate the toxicity profile in animal models.

Thus this study will help in further extension of the therapeutic potential of Rathi nagara rasa mezhugu (RNM).

2. AIM AND OBJECTIVES

AIM

To evaluate the Preclinical safety profile of *Rathi nagara rasa mezhugu (RNM)* – a herbo-mineral preparation.

OBJECTIVES

1. To analytical evaluation of *Rathi nagara rasa mezhugu*
2. To study the toxicity profile of *Rathi nagara rasa mezhugu* .

SECONDARY OBJECTIVES

1. Literature of review
2. Preparation of *Rathi nagara rasa mezhugu* .
3. To do the following studies on *Rathi nagara rasa mezhugu*
   - Physico-chemical analysis and Phyto- chemical analysis
   - Biochemical analysis
   - Heavy metal analysis by using ICP-OES
   - Particle size analysis by using SEM
   - Acute Oral Toxicity study as per OECD guideline-423
   - Repeated 28-days Oral Toxicity study as per OECD guideline-407

இரசம்

வேறு பெயர்கள்:

தசாங்க நிகண்டில் கூறியவை:

காரம், சூதம், புண்ணியம், கற்பம், சாமம், சத்து, சூரியவிரோதி, சாதி, சூத்திரன், துள்ளி, ஈசன், வீரியம், சூழ்ச்சி, நீர், விண்ணினீர், விண்மருந்து, இரதம், சுக்கிலம், போகம், மூலம், சிந்தூரம், சிந்து, பக்கிரம், பதினெண்பந்தி, பாரதம், கனல், பூதம்.

சட்டமுனி நிகண்டில் கூறியவை:

இனிமை, சிவசக்தி, வருணத்தோன், தனிமை, சங்கரன்விந்து, பனிமை, பராபரம், பாய்ந்திடுதூமம், கனிமை, சரக்கிற்கலந்திடுசீவன், சிவன்விந்து, காவன், சிதறிக்காண்போன், கேசரி, வேந்தன், பாவன், அந்தரகந்தன், ஆதி, வராட்டியன், சுந்தரம், சொற்குறி, தூமம், மகாமரம், மந்தரம், மஞ்சி, மாருதம், மகிபன், விந்தரம், சிலை, கணவன், மலைக்குறவன், வாசுகிநாதன், கந்தன், காவக்குடியோன்.

இரசத்தின் பிரிவுகள்:

1. இரசம்
2. இரசேந்திரன்
3. சூதம்
4. மிசரகம்
5. பாரதம்

சுவை:

அறுசுவை, சிறப்பாய் இனிப்பு.

வீரியம்:

வெப்ப சீத வீரியங்கள் இரண்டும் கொண்டது.

பிரிவு:

எப்பொருளை இதற்குத் துணைமருந்தாக்கிக் கொடுக்கின்றோமோ அப்பொருளின் பிரிவை இது அடையும்.

செய்கை:

- உடல்தேற்றி
- உடல்உரமாக்கி
- மலம்போக்கி
- பித்தநீரகற்றி
- வீக்கமுறுக்கி
- உமிழ்நீர்பெருக்கி
- சிறுநீர்பெருக்கி
- மேகநாசினி

மகிமை

இது சீதத்தால் உண்டாகும் நோய்கட்கன்றி வெப்பத்தால் உண்டாகும் பிணிகட்கும், உள்ளாட்சிக்கன்றி வெளியாட்சிக்கும் எல்லாவற்றிற்கும் தலைசிறந்தது. இரசம் முங்கூறப்பட்டது போன்று வெப்பம் அல்லது சீத வீரியங்களை மட்டுமுடைய மற்ற பொருள்களை போலன்றி வெப்ப சீத வீரியங்களை சம அளவிலுடையது. ஆகையால் எவீரியத்தையுமுடைய பொருள்களோடு சேர்கின்றதோ அதன் வீரியத்தையும் செய்கையும் அடையும் தன்மைவாய்ந்த்தது.

பாதரசத்தின் பொதுகுணம்:

"விழிநோய் கிரந்திகுன்மம் மெய்ச்சூலை புண்குட்
டழிகாலில் விந்துவினால் அத்தை – வழியாய்
புரியு விதி யாது புரியினோ யெல்லாம்
இரியுவிதி யாது மில்லை"

பொருள்:

சிவ வீரியம் எங்கின்ற இரசத்தை முறைப்படி மருந்தாக்கிக் கொடுக்க அது கண்ணோய், கிரந்தி, எண்வகைகுன்மம், சூலை, பெரும்புண், தொழுநோய், வளிக்குறைவு முதலிய நோய்களை நீக்கும்.

நற் குணம்:

- குருதியைச் சுத்தி செய்து துர்நீரை நீக்கல்
- குருதியையும் சுக்கிலத்தையும் பெருக்கல்
- பசியைத் தூண்டல்
- கிருமிகளைக் கொன்று புண்புரைகளை ஆற்றல்
- உடல்முழுவதையோ அல்லது உள்ளும் புறமுமான் உறுப்பின் பகுதியையோ உறுப்பின் முழுவதையோ பற்றிய வியாதிகளை குணமாக்கல்
- முக வசீகரத்தை உண்டாக்கல்
- மறதியை ஒழித்து மூளைக்கு கவன சக்தியைத் தரல்
- நரம்புக் கூட்டங்களை வன்மையுறச் செய்தல்
- மனதை ஒரு நிலையில் நிறுத்தி ஞானத்தை விருத்தி செய்தல்
- மூப்பை ஒழித்து ஆயுளை வளர்த்தல்

தீக் குணம்:

இரசத்தை சரியான முறையில் முடித்து உண்ணாத்தால் வரும் குற்றங்கள் அநேகம்.

இரச தோடம்:

இரச தோடம் 2 வகை

1. 8 வகை தோடம்
2. 7 வகைசட்டை

இரச சுத்தி முறைகள்:

ஒரு பலம் இரசத்திற்கு தும்பை சமூலச்சாறு (4¾பலம்) விட்டு சூரியபுடம் வைக்கவும். இவ்வாறே 10 நாட்களுக்கு நாள்தோறும் புதிய சாறு விட்டு வெய்யிலில் வைக்கவும். பிறகு சாறு விடாமல் முற்றிலும் சுவறும்படி வெய்யிலில் வைத்தெடுக்கவும். இம்முறையை மேலும் ஒரு முறை செய்யவும். இவ்விரசத்தையும் இரசத்துடன் உள்ள தூளையும் ஒரு மட்பாண்டத்திலிட்டு 2படி தும்பைச்சாறு விட்டு கவசம் செய்து பூமியில் 20நாள் புதைத்து எடுக்கவும் .பிறகு நீர் விட்டு கழுவி எடுக்கவும். இவ்வாறு சுத்தி செய்த இரசம், பற்பம், செந்தூரம், குரு, குளிகை முதலியவற்றிற்கு ஆகும்.

ஒரு பலம் இரசத்தை செங்கல் மாத்தூளிலும் மஞ்சள்பொடியிலும் ஒவ்வொரு மணிநேரமும் ஆட்டி சுத்தநீரில் அலம்பி, ஒரு படி மேனிச் சாற்றிலிட்டு அடுப்பேற்றி சாறு சுண்டும் படி எரித்து எடுக்கையில் சுத்தியாகும்.

5பலம் இரசத்திற்கு 1பலம் சித்திரமூல வேர்ப்பட்டைத் தூள் 1பலம் திரிகடுகு பொடி, 1பலம் காயம், 1பலம் இந்துப்புத்தூள் , இத் தூள்களை சிறிது சிறிதாய் சேர்த்துக் கல்வத்திலிட்டு 36மணி நேரம் அரைத்து எடுக்க பின்பு 1குழல் கொண்டு தூள்களை ஊதிநீக்குக். இவ்விதம் 7 முறை செய்ய இரசம் உயர்தர சுத்தியாகும்.

வேண்டிய அளவு இரசத்தை தூயகெட்டித் துணியிலிட்டு 1000முறை பிழிந்தெடுக்கவும். இதை ஒரு மட்சட்டியிலிட்டு அதன் மீது ஒரு அங்குலமிருக்கும் படி சுத்த நீர் விட்டு சிறுதீயில் எரிக்கவும். நீர் குறையாமல் அந்த அளவைகாத்துக் கொள்ளவும். நீர் கருநிறம் அடைந்தவுடன் எடுத்தநீரை காடிநீரில் நான்கு அல்லது ஐந்து முறை கழுவி எடுக்கபூரண சுத்தியாகும்.

விதை நீக்கின மிளகாயில் இரசத்தை விட்டு கோவையிலையை அரைத்து 4விரற்கடை கன்ம் கவசித்து சீலைமண் 7 செய்து குக்கிடப்புட மிட்டு எடுக்கையில் சுத்தியாகும்.

பத்தியம்:

இரச சம்பந்தமான மருந்துகள் அருந்துங் காலத்து மீன், உப்பு, மிகுசீதம், மிகுவெப்பம், மந்தப் பொருள், எண்ணெய், மதுபானம், கைப்பு, கார்ப்பு, புளிப்பு சுவையுள்ள பொருள்கள், பெண் போகம் ஆகா[7].

இரச நஞ்சுப் பொது குறி குணங்கள்:

தூய்மை செய்யாததும் நன்றாக முடிக்கப் பெறாததும், அளவுக்கு அதிக மானதுமான இரசத்தை உண்பதனால் குற்றம் உண்டாகும். வாயில் அச்சரத்தைப் போல் புண் உண்டாகும், பனங்கள்ளைப்போல் வாய் குழம்பும், வாய் தொண்டை இவை வெந்து வீங்கும். வயிறு, குடல் இவைகள் புண்ணாகும். பக்கத்தில் அடிக்கடி வலித்துக் கொண்டே இருக்கும். வயிற்றின் மீது பட்டை பட்டையாக தேமல் படரும். காலடியில் வெடிப்பு கண்டு விட நீர் கசியும். நீரடைப்பு, இரத்தபேதி, உடல் வெளுத்தல், காது செவிடுபடல், கண் பார்வை இழத்தல், சிரங்கு, புண் செம்படை போல் உடலில் படை உண்டாதல் ஆகிய குறிகுணங்கள் உண்டாகும். மேலும் பித்தன் போன்று வாய்பிதற்றும், துணிகளை கிழித்து அவிழ்த் தெறியச் செய்யும். கல்லால் அடிக்கவும், மலையேறிக் குதிக்கவும், தண்ணீரில் விழுந்து முங்கி முங்கி வெளிவரவும், வியர்வை பெருகும் ஆகிய குறிகுணங்களைக் காட்டும்.

இரச நஞ்சு முறிவு:

- குண்டியை பிடித்தக்கால் சாயப்பட்டையைப் பொடித்து வெல்லத்துடனே கலந்து கொடுக்க வேண்டும்.
- பல்லுக்கிட்டினால் கோவைத்தண்டுச்சாற்றை நாக்கில் பிழியத் தீரும்.
- நெஞ்சுவற்றி, உள்வெதும்பி, மேல்எரிவுஎடுத்து, கை கால் மண்டி எரிந்து நினைவ்வில்லாமல் கிடந்தால் அறுகங்கிழங்கை ஆய்ந்து எடுத்து அரைத்து வெள்ளாட்டுப்பால், பசுவின்பால். பருத்திக்

கொட்டைப்பால், மோர், இவைகள் ஏதாவதொன்றில் கரைத்து வடிகட்டிக் கொடுக்க வேண்டும்.

- வெள்ளை முட்சங்கன் இலைச்சாறு அல்லது மிதிபாகலிலைச் சாறு இவைகளின் ஏதாவதொறு சாற்றை 80மி.லிவீதம் காலையிலும் மாலையிலும் 3நாள் கொடுக்க வேண்டும்.
- அவுரிவேர்ப்பட்டையை வெந்நீர் விட்டரைத்து சுண்டைக்காய் அளவு காலையிலும் மாலையிலும் 3 நாள் கொடுக்க வேண்டும்.
- துளசி வேர்ப்பட்டைக் குடிநீர்
- கரு வேற் கொப்புளிக் குடிநீர்
- எருக்குக் கொப்புளிக் குடிநீர்
- சுரைக் கருப்பு
- தயிர் வெல்லம்[8]

சேரும் மருந்துகள்:

- குப்பிச் செந்தூரம்[9]
- சுயமாக்கினி செந்தூரம்[10]
- சுர மாத்திரை[10]
- சந்திரோதயக் குளிகை[11]
- பாடாண மாத்திரை[11]
- இரசப் பற்பம்[12]
- இரசச் செந்தூரம்[12]
- திரிநேத்திர மெழுகு[12]
- சஞ்சீவி குழம்பு[13]
- பரஞ்சோதி மாத்திரை[13]
- மலைக்காத்தான் குளிகை[13]
- சகல விடத்திற்கு இரசத்தின் மை[14]
- விஷக்கடிக்கு நாபிக் குழம்பு[14]
- விஷக்கடிக்கு இரசாதிக் குழம்பு[14]

MERCURY

Physical appearance:

Metallic mercury is a heavy silvery liquid and is not poisonous, but it volatilizes at room temperature and inhalation of the vapor is highly toxic.

Derivatives:

- Mercuric chloride.
- Mercurous chloride
- Mercuric sulphide.
- Mercuric oxide.

Mercury in any form is toxic. There are 3 forms of mercury.

- Elemental.
- Inorganic.
- Organic

Elemental Mercury:

It is found in liquid form and easily vapourises to a monatomic state at room temperature.

Inorganic Mercury:

Found mostly in salt form, it is highly toxic and corrosive.

Organic Mercury:

It is lipid soluble, and mild corrosive.

Absorption:

The main route of absorption (80%) of elemental mercury through respiratory tract. It is due to its monatomic form and lipid solubility. But it is poorly absorbed in GI tract and is mildly toxic through ingestion as it occurs as larger globular particles. Inorganic mercury gains entry in the body either orally or dermally. Organic mercury is absorbed completely in GI tract.[15]

Distribution:

Mercury as a heavy metal tents to accumulate in the lowest part of the body, such as floor of the mouth, pelvic floor and feet

(Alternative medicine magazine 1997)

Mercury is a toxic metal widely occurring in the biosphere which presents hazards associated with both ingestion and inhalation.

Pesticides, large fish and mercury dental filling are the most potent sources of mercury.

Mercury has also been used as an antiseptic and pesticide. Many commercial preparation have contained the inorganic mercury salt calomel (Mercurous chloride), including over the counter laxative preparations and some cosmetics.

The largest source of mercury for most people in the western world amalgam (Silver) dental filling. The normal chewing of food cause the abrading of amalgam from the filling leading to the ingestion of small particles of mercury. Natural endogenous bacteria of the mouth and gut are able to convert inorganic mercury into organic mercury through methylation. (Adding a methyl group to the mercury element) and so forming methyl mercury.

The WHO states that the largest estimated average daily intake and retention of mercury and mercury compounds in the general population Is from dental amalgam filling. The estimated daily intake of mercury from dental amalgams is 3.8 – 21 mcg per day.[16]

Uses:

1. Medicinal Uses:

- Disinfectant.
- Dental filling.
- Diuretic.
- Treatment of syphilis.

2. **Industrial Uses:**

- Thermometer.
- Barometer.
- Calibration instruments.
- Fluorescent and mercury lamp.

3. Miscellaneous:

- Golden silver extraction
- Photography
- Insecticide and pesticide
- Embalming [16]

TOXICOLOGICAL ASPECTS

Acute toxicity:

Ingestion:

Metallic taste, burning pain in the mouth extending down to the stomach and abdomen followed by nausea, vomiting and then diarrhea. The urine is suppressed or scanty.

Inhalation:

Metallic taste, salivation, gingivitis and loosening of teeth, lethargy, slurring of speech, diarrhea, pneumonitis, cough, cyanosis and anuria.

Chronic toxicity:

1. Hydragyrism:

Salivation,Metallic taste, steatites, gingivitis, bluish black gum line (Burtonian line), loosening of teeth.

2. Tremer:

The advanced condition called "***Hatter's shake***" the person then becomes unable to dress him write legibly or walk properly.

3. Mercurialentis:

Discoloration of the lens of the eye when viewed through a slit lamp.

4. Acrodynia:

Mercurous chloride used to be given to children in the past as teething powder. This gave rise to a condition called acrodynia (pink disease) characterized by fever, skin rash, joint pain and spleenomegaly. The cheeks, tip of the nose, hands and feet appear intensely pink.[17]

Biological effects:

Mercury has toxic effects on numerous organs and system. Themajor target organs being CNS and kidney. Both organic and inorganic compounds have an avid affinity for thiol chemical groups and this is the property, which send them toxic. Most mercury compounds are potent but unspecific inhibitors affecting membrane permeability, nerve conduction and tissue respiration. In this aspect biochemical effects of mercury resemble those of **"black widow spider venom".**

Highest concentration of mercury occurs in kidney regardless of chemical form absorbed. Kidney is the primary target organ only in the case of inorganic mercury.

Renal tissue contains a thiol rich protein called metallothionein. Exposure to toxic metals trigger the production of protein,which binds tightly to the metal retaining in kidney in relatively harmless form. As long as the kidney's capacity for the production of metallothionein is not overwhelmed, mercury excretion eventually balance intake, thereby limits worsening of symptoms. However, acute high dose or increase in chronic dose level Precipitates renal failure, which is a classical symptom of mercurial poisoning. Massive oral doses of inorganic mercury initiates a chain of events with anural progressing to polyuria then finally recovery to normal renal function.

More useful form mercurial toxicity occurs with chronic industrial exposure, which is characterized by polyuria. If severe, nephritic syndrome is observed.[18]

Toxic symptoms of mercury in various systems:

If a person has seven or more of these symptoms, there is a significantly increased possibility that mercury toxicity is a major contributing factor.

Central nervous system:

- Irritability.
- Anxiety/ nervousness, often with difficulty in breathing.
- Restlessness.
- Exaggerated response to stimulation

- Fearfulness.
- Emotional instability:
    1. Lack of self control.
    2. Fits of anger, with violent, irrational behaviour
- Loss of self confidence
- Indecision.
- Shyness or timidity, being easily embarrassed
- Loss of memory.
- Inability to concentrate.
- Lethargy, drowsiness.
- Mental depression, despondency
- Suicidal tendencies.
- Manic depression.
- Numbness and tingling of hands, feet, fingers, toes or lips.
- Muscle weakness progressing to paralysis.
- Ataxia
- Tremors/trembling of hands, feet, eyelids or tongue.
- In coordination
- Myoneural transmission failure resembling myasthenia gravis.
- Multiple sclerosis.

Head, Neck, Oral cavity disorders:

- ✓ Bleeding gums.
- ✓ Alveolar bone loss.
- ✓ Loosening of teeth.
- ✓ Excessive salivation
- ✓ Foul breath
- ✓ Metallic taste
- ✓ Burning sensation, with tingling of lips, face.
- ✓ Tissue pigmentation (amalgam tattoo of gums)
- ✓ Leukoplakia.
- ✓ Ulceration of gingivitis, palate, tongue.
- ✓ Dizziness.
- ✓ Hearing difficulties.

Gastrointestinal effects:

- ❖ Food sensitivities, especially to milk and eggs.
- ❖ Abdominal cramps, colitis, diverculitis or other gastro intestinal complaints.
- ❖ Chronic diarrhea / constipation.

Cardiovascular effects:

- ➢ Abnormal heart rhythm
- ➢ Characteristic finding on ECG
- ➢ The Abnormal changes in the S-T segment and / or lower
- ➢ Broadened P wave.
- ➢ Unexplained elevated serum triglycerides.
- ➢ Unexplained elevated cholesterol.
- ➢ Abnormal blood pressure, either high or low.

Systemic effects:

- ❖ Chronic headaches
- ❖ Allergies
- ❖ Severe dermatitis
- ❖ Thyroid disturbances
- ❖ Cold, clammy skin, especially handsome feet
- ❖ Chronic kidney disease.
- ❖ General fatigue.
- ❖ Loss of appetite.
- ❖ Loss of weight.
- ❖ Hypoglycemia.[18]

Mechanism of toxicity:

Mercury can be found in a two main forms, inorganic and organic. Inorganic mercury is very toxic to humans, but not nearly as toxic as organic mercury such as methyl mercury. Methyl mercury is a form of mercury which has been bound to a simple organic carbon group. This makes it permeable to membranes and encourages its movement into brain tissues. About 10% of mercury ingested accumulates in the brain.

Mercury has an affinity for organic sulphur compounds called ***thiols,*** which are essential compounds of enzyme systems. Mercury will irreversibly blind to these thiol groups, and inhibit their function in enzyme reactions, Thiols are also involved in protein formation and help stabilize protein structure. Mercury is then able to cause the denaturation of protein structures particularly in the brain. It can also form a hapten with the protein it is bound to causing the immune system to recognize that protein as foreign particle destroying it at all opportunities. This leads to beginning of autoimmune disorders.

Mercury detoxification:

In attempt to reverse the problem of mercury toxicity it is important to realize that the mercury contamination must be removed.

Whether this is the cessation of using cosmetics, eating fish or having dental amalgams removed.

It is also important to supplement those nutrients most affected by mercury as this appears to one way of reducing the effects of chronic exposure. High protein diet is recommended. As Sulphur bearing amino acid in protein will greatly facilitate detoxication, as mercury will attach to sulphuramino acids in protein, it is important to supplement with nutrients to encourage mercury elimination.

Glutathione (USH):

Glutathione is an important antioxidant amino acid which protects against mercury toxicity.

N-acetyl L-cysteine (NAC):

In experiments where animals were exposed to mercury vapor, NAC treatment increased, animal survival time and decreased the mercury levels in blood, lung and kidney tissues from these animals.

L.Methione:

L.Methione is an essential sulphur containing amino acid. It is used by the body to produce cysteine, cystathionine, glutathione and taurine. Mercury is able to

bind to methionine and inhibit it being used in the production of cysteine and glutathione**.**

Zinc:

Mercury is able to compete with displace zinc in a number of biological systems. Thus mercury causes zinc deficiencies in various tissues such as the brain. Zinc stimulates the production of metallothionein is very rich in cysteine. Supplemental zinc is therefore vital in any mercury elimination programme.

Ascorbic acid (Vitamin C):

Prolonged exposure to mercury tends to depress the adrenal. Ascorbic acid content providing vitamin C should help restore and or maintain adequate adrenal levels of this critical nutrient, thus offsetting the depletion of this chemical due to streeses caused by chronic inhalation of mercury vapor.

Selenium:

This essential mineral is able to bind to mercury and is able to cause a redistribution of tissue mercury. It is therefore able to precipitate the excretion of some mercury from the body.

Garlic:

Garlic's powerful actions come from its sulphur contains constituents, including allicin, allin, and daallydisulphidde. These comounds are quite capable of binding to and eliminating mercury as a normal part of their physiological action.

A number of other nutrient help support. These include thiamine (Vitamine B1), magnesium, potassium and chromium. Fatigue is a common symptom of mercury toxicity and one that can e alleviated with aid of Ginko Biloba, cats claw (Uncaria tomentosa) and Co enzyme Q10, all of these nutrients are capable of increasing energy levels in the body, following the patient to feel increased vigour.[16]

The circumstances of poisoning:

Accidental poisoning by mercuric chloride may be due to the use of strong solution in washing abscess cavities or irritating vagina, uterus or rectum. Homicidal and suicidal poisoning is rare.

Post mortem appearances:

The mucosa of GI tract shows inflammation, congestion, corrosion, acute tubular and glomerular degeneration or heamorrhagic glomerular nephritis is seen. The liver is congested and shows cloudy swelling or fatty change. [19]

கந்தகம்

வேறுபெயர்:

காரிழையின்நாதம், பரைவீரியம், அதீதப்பிரகாசம், பீஜம், செல்விவிந்து, சக்தி, சத்திபீசம், செந்தூரத்தாதி, தனம், தேவியுரம், நாதம், நாற்றம், பரைநாதம், பொன்வர்ணி, இரசசுரோணிதம்.

பாடாணங்கள் அறுபத்து நான்கில் பிறப்புகந்தகம், வைப்புகந்தகம், கோழித்தலை கெந்திவைப்பு, வாணகெந்தி வைப்பு என்று நான்கு பாடாணங்கள் கூறப்பட்டுள்ளது.

கந்தகத்தின்வகைகள்:

வெண்மை நிறம்	-	எல்லா நோய்களையும் தீர்க்கும்
கிளி மூக்குச் சிவப்புநிறம்	-	நவலோகத்தை ஏமமாக்கும்
பொன்மை நிறம்	-	குற்றமற்ற நெல்லிக்காய் போன்று இருக்கும், சூதகத்தோடு உறவாகி சுத்தமாய் இருக்கும்
காகத்தின் நிறம்	-	நரை திரை அற்றுப்போகும்

பதார்த்த குண சிந்தாமணியில் நெல்லிக்காய் கந்தகம், வாண கந்தகம் ஆகியவற்றின் குணங்கள் கூறப்பட்டுள்ளன. மருந்துகளில் கையாளப்படுவது நெல்லிக்காய் கந்தகமாகும்.

நட்புச்சரக்கு:

“கந்தகத்தின முமிரசந்தா னென்றாரே”

கந்தகத்தின் நட்புச்சரக்கு ரசம்.

பகைச்சரக்கு:

"சொல்லுமே தாம்பிரத்தைக் கெந்திகொல்லும்"

கந்தகந்தின் பகைச்சரக்கு செம்பு.

சுவை:

கைப்பு, துவர்ப்பு

செய்கை:

- மலமிளக்கி
- உடல்தேற்றி
- வியர்வைபெருக்கி
- கிருமிநாசினி
- பித்தநீரை அதிகப்படுத்தும்
- அசுகங்களின் சளிச்சவ்விலுள்ள கோளங்களின் சுரப்பை அதிகப்படுத்தும்.
- விரேகியில் சிறப்பாக செயல்பட்டு சுரப்பை அதிகப்படுத்தும்.

நெல்லிக்காய்கந்தகத்தின்குணம்:

"நெல்லிக்காய்க் கெந்திக்கு நீள்பதினெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் – பொல்லா
விடக்கடுவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரக ணிகபம்போந் தேர்"

– பதார்த்த குண சிந்தாமணி

பொருள்:

பதினெண்குட்டம், மந்தம், கல்லீரல்வீக்கம், குன்மவாயு, கண்ணோய்கள்,கொடுமையை செய்கின்ற விடக்கடிகள், நாட்பட்ட மேக நோய்கள், வாதசுரம், பேதி, நாட்பட்ட கிரகணி, கபம் முதலியன நீங்கும்.

வாணக்கந்தகத்தின்குணம்

"வாணக் குழாய்கந்தி வாசனையைக் கண்டவுடன்
காணக் கிருமி சொறி காணாவாம் – தோணும்
பெருவியா திக்கூட்டம் பேருமத னூலின்
மருவியா ழுங்கொடியே வாழ்த்து"

பொருள்:

வாண மருந்துக்கான குழாய்கந்தகத்தின் வாசனையைக் கண்டவுடன் இரச இரத்தத் தாதுக்களில் பிறந்தக் கிருமிகள், சொறி, குறைநோய்க் குட்டங்கள், நாட்பட்ட கீல் வாதம், சுவாசகாசம், மாரடைப்பு, கண்டமாலை, குதநெகிழ்ச்சி போன்ற நோய்கள் நீங்கும்.

"மாதர் மகவை வளர்ப்பதுபோ லேயுடம்பை
யாதரவா கத்தேற்றி யாக்கையினால் – மீதாக
மேவி யடர்நோயின் வெப்பத்தை மாற்றுதலாற்
றேவியுர மென்பதுடல் தேர்"

– தேரன் பொருட்பண்பு நூல்

பொருள்:

கந்தகம் தாய் மகவை வளர்ப்பது போல நோய்களின் வெப்பத்தை மாற்றி உடம்பைத் தேற்றுவிக்கும்.

சுத்திமுறைகள்:

- கந்தகத்தை ஒரு இரும்புக்கரண்டியில் இட்டு சிறிது பசுவெண்ணெய் இட்டு உருக்கிப் பசும்பாலில் சாய்க்கவும்.இவ்விதம் 30 முறை செய்ய கந்தகம் சுத்தியாகும்.ஒவ்வொரு முறையும் புதியபாலையே உபயோகிக்க வேண்டும்.

- பாலுக்கு பதில் வாழைக்கட்டைநீரில் கந்தியைப் பத்துமுறை உருக்கி உருக்கிச் சாய்த்து எடுக்கு சுத்தியாம்.இம் முறை கந்தகத்தில் உள்ள எண்ணெய் நீங்கும்.
- மருதோன்றிக் கற்கத்தை பசுவின்தயிரில் கலந்து ஒரு சட்டியில் இட்டு சீலையில் வேடுகட்டி, அதன் மேல் கந்தகத்தை வைத்து மற்றொருசட்டியால் மூடி சீலை செய்து குழியில் புதைத்து, மேல்சட்டி மேல் 5 வறட்டி கொண்டு புடமிட கந்தகம் உருகிக் கீழே இறங்கும். இவ்விதம் 7 முறை செய்ய வேண்டும்.
- புளியம்பழ ஓட்டைப் பற்றியிருக்கும் கசிவை ஊறவத்துஇறுத்த நீர், காடிநீர், புளித்தமோர், காளான்சாறு இவைகளை தனித்தனி 6 பலமாக எடுத்துக்கலந்து ஒரு சட்டியிலிட்டு அச்சட்டிக்கு சீலையினால் வேடுகட்டி அதன்மேல் ஒருபலம் கந்தகத்தை வைத்து மேல்மூடி அடுப்பேற்றி தீபாக்கினியாய் 2 சாமம் எரிக்க மலினம் மேல் தங்கி கந்தகம் சுத்தியாகிக் கீழிறங்கும்.

அளவு:

10 (650மிகி) முதல் 30 (1.9கி) உளுந்தெடை[7]

சேரும் மருந்துகள்:

- கந்தக தைலம்[9]
- சுயமாக்கினி செந்தூரம்[10]
- கந்தக ரசாயணம்[20]
- சந்நிபாத பைரவம்[21]
- கந்தக செந்தூரம்[22]
- சண்டமாருத செந்தூரம்[23]
- மால்தேவி செந்தூரம்[23]
- கந்தக மெழுகு[24]
- ஆறுமுக செந்தூரம்[25]
- நாதகுரு தைலம்[25]

SULPHUR

Sulphur is a chemical element with symbol S and atomic number 16. It is an abundant, multivalent non-metal. On Earth, elemental sulfur can be found near hot springs and volcanic regions in many parts of the world, especially along the Pacific Ring of Fire; Native sulfur is synthesized by anaerobic bacteria acting on sulfate minerals such as gypsum in salt domes. Common naturally occurring sulfur compounds include the sulphide minerals, the sulphates and barite.

SOURCE:

A non metallic element found free in beds of gypsum and in a state of sublimation in regions of extinct volcanoes; also in combination with several ores called pyrites, as sulphates and sulphides of iron, copper, lead, zinc, mercury, etc.

REGIONAL NAMES:

Tamil	:	Gandhagam
English	:	Brimstone; sublimed sulphur
Hindi	:	Gandak
Sanskrit	:	Gandhaka

PHYSICAL PROPERTIES:

Sulphur forms polyatomic molecules with different chemical formulas, with the best-known allotrope being octasulphur, cyclo-S8. Octasulphur is a soft, bright-yellow solid with only a faint odor. It melts at 115.21 °C (239.38 °F), boils at 444.6 °C (832.3 °F) and sublimes easily. All of its stable allotropes are excellent electrical insulators.

CHEMICAL PROPERTIES:

Sulphur burns with a blue flame concomitant with formation of sulphur dioxide, notable for its peculiar suffocating odor. Sulphur is insoluble in water but soluble in carbon disulphide and, to a lesser extent, in other nonpolar organic solvents, such as benzene and toluene.

Sulphur in blood:

Total level – 3.4 mg%. it exists in three distinct forms.

A) Inorganic sulphate – 1 mg %
B) Neutral sulphate -1.9 mg%
C) Ethereal sulphate -0.5 mg%

ACTION:

Sulphur is described as of bitter astringent taste with a peculiar strong smell. It increases bile, act as a laxative and alternative and its preparations also act as alternative, laxative, diuretic and insecticide. Sulphur, when taken internally and in small doses, becomes absorbed and may be detected in the sweat, milk and urine. It is a stimulant to the secreting organs such as the skin and bronchial mucous membranes. It has a specific action on the rectum and increases the hemorrhoid secretions. The sulphurous and mineral waters as they contain earthy and Alkaline sulphates act as laxative and diuretic, while the sulphurous acid disengaged from them acts as a diaphoretic. In large doses it acts as a purgative.

USES:

It readily combines with and fixes metallic mercury and is therefore extensively used in combination with that metal. In combination with cream of milk, sulphur is given in diseases like hemorrhoids, prolapsed and stricture, also in chronic skin diseases; in skin diseases sulphur is used both internally and externally. Sulphur is useful in cough, asthma, consumption and general debility; also useful in enlargement of the liver and spleen, chronic fevers etc. As sulphur is a mild laxative, for habitual constipation, in the presence of hemorrhoids, equal parts of sublimed sulphur and cream of tartar with a little honey or milk in doses of 1 drachma is taken before each meal. Dose is half to one teaspoonful once or twice daily. This also acts beneficially in cases of piles and chronic dysentery. A sulphur bath is generally efficacious for skin diseases, as itch, acne, rosacea, sycosis and chloasma and internally sulphur powder or mineral sulphureted waters are given with benefit. In many households sulphur is used to disinfect rooms by fumigation. In cases of chronic rheumatism a liniment composed of two ounces of powdered or sublimed sulphur and a pint of neem oil well rubbed in, twice daily, is very beneficial.[26]

METABOLISM AND BIOLOGICAL FUNCTION:

Sulphur is metabolized by all organisms, from bacteria to plants and animals. Sulfur is reduced or oxidized by organisms in a variety of forms. The element is present in proteins, nucleic acids, sulfate esters of polysaccharides, steroids, phenols, and sulfur containing coenzymes. Sulfur is an essential element for all life, and is widely used in biochemical processes. In metabolic reactions, sulfur compounds serve as both fuels and respiratory materials. Sulfur in organic form is present in the vitamins biotin and thiamine. Sulfur is an important part of many enzymes and in antioxidant molecules like glutathione and thioredoxin. Organically bonded sulphur is a component of all proteins, as the amino acids cysteine and methionine. Disulphide bonds are largely responsible for the mechanical strength and insolubility of the protein keratin, found in outer skin, hair, and feathers.[27]

PHARMACEUTICALS:

Sulfur (specifically octa sulfur, S8) is used in pharmaceutical skin preparations for the treatment of acne and other conditions. It acts as a keratolytic agent and also kills bacteria, fungi, scabies mites and other parasites. Precipitated sulfur and colloidal sulfur are used, in form of lotions, creams, powders, soaps, and bath additives, for the treatment of acne vulgaris, acne rosacea, and seborrhoeic dermatitis. [28,29]

Common adverse effects include irritation of the skin at the application site, such as dryness, stinging, itching and peeling.

TOXICOLOGICAL ASPECT OF GANDHAGAM:

Elemental sulfur is non-toxic, as generally are the soluble sulfate salts. Soluble sulfate salts are poorly absorbed and laxative. However, when injected parent rally, they are freely filtered by the kidneys and eliminated with very little toxicity in multi-gram amounts.

When sulfur burns in air, it produces sulphur dioxide. In water, this gas produces sulfurous acid and sulfites, which are antioxidants that inhibit growth of aerobic bacteria and allow its use as a food additive in small amounts. However, at high concentrations these acids harm the lungs, eyes or other tissues.[30]

சேராங்கொட்டை

வேறுபெயர்:

சேங்கொட்டை ,வல்லாதி, வல்லதாகி, எரிமுகி, பல்லதாகி, கிட்டாக்கனிக் கொட்டை, நந்திவித்து.

பயன்படும்உறுப்பு:

கொட்டை, பருப்பு

சுவை:

கைப்பு, விறுவிறுப்பு

தன்மை:

வெப்பம்

பிரிவு:

கார்ப்பு

செய்கை:

உடற்தேற்றி, புண்ணாக்கி

குணம்:

“குட்டங்கய ரோகங் கொல்லும் விடபாகந்
துட்டந்தரு கிருமி சூலையும் போம் – மட்டலருங்
கூந்தன் மயிலே கிரந்திக் கூட்டம் போஞ் செங்கையில்
ஏந்து சேங்கொட்டை தனையே”

– அகத்தியர் குண வாகடம்

பொருள்:

பெருநோய், இளைப்புநோய், நஞ்சுகள், சூலை, திமிர்படை, கரும்படை, வெண்குட்டம், தீராக்கடி, மூலம், வளிநோய்கள், குன்மம் இவைகளை போக்கும்.

குணம்:

"சேரா தழகு வடிவினுக்குச் சேரு மதிகப் பசியுண்டாம்
சேங்கன் றென்ன வுரமுண்டாஞ் சேகண்டியைப் போல்
தொனி யுண்டாம்
சேட னெனவே யின்பமெலாஞ் சேர்ந்தே அணைய மென்மேலும்
சேயின் முகம்போல் களையுண்டாம் சேர்க்கே தனன்போல்
எழிலுறுமே"

– தேரன் கரிசல்

பொருள்:

சேராங்கொட்டை லேகியத்தை முறைப்படி கொள்ளின் அழகும் பசியும் உண்டாம்.செங்கன்றை யொத்த உரம்எழும் ,செய்பேரிகையின் ஒலியைப் போல் குரல்ஒலி பெருகும். ஆதிசேடனைப் போல் இன்பந் துய்க்க வலிவு உண்டாகும், குழந்தையின் முகம் போல்களையும், மன்மதன் போன்ற எழிலும் உண்டாகும்.

காற்பாச மாகக் கடுப்புகா லின்முன்னே
காற்பாச மாகக் கதறுமே- காற்பாசஞ்
சேரா விரைக்கற்பஞ் சீராவா மெய்க்கதனாற்
சேரா விரைக்கற்பந் தின்

– தேரன் யமக வெண்பா

பொருள்:

இதனை கற்பமாகக் கொள்ள, காலிலே விலங்கிருப்பதைப் போன்று நடக்கவொட்டாமற்படி கால்களை முடக்கும் வளிநோய்களும், அதனால் உண்டாகும் குத்தலும் பெருங்காற்றை எதிர்த்த பஞ்சாகும்.

சுத்திமுறைகள்:

- சேங்கொட்டை மேல் மரம்போல் உள்ள மூக்கை சீவி, சுண்ணாம்புக்கு நடுவில் வைத்து கள் அல்லது காடிவிட்டு தாளித்துக் கழுவி எடுக்கவும் .இவ்வாறாக ஆறு முறை செய்யத் தூய்மையாகும்.
- புளியிலை, புரசம்பூ இவைகளின் குடிநீரிலும், பசுஞ்சாணப்பாலிலும் கற்றாழைச்சாற்றிலும் முறையே வேகவைத்துஎடுக்கலாம்.
- சேராங்கொட்டை ஓரளவு, இரட்டையளவு பனங்கள்ளும், சிகப்பு பசுந்தயிறும் கூட்டி ஒண்பது நாள் சூரியனில் காய்ந்த பின் கழுவி எடுத்துக் கொள்ள வேண்டும். இம் முறை தேரன் கரிசலில் கூறப்பட்டுள்ளது.[31]

நஞ்சுக் குறிகுணம்:

உடம்பில் பட்ட மாத்திரத்திலே வேக்காடை உண்டாக்கி புண்ணாக்கும். மருந்து செவ்வனே பக்குவப் படாவிட்டால் உட்கொண்டவுடனே வாய், வயிறு, குடல் முதலியன்ப் புண்ணாகும். சில சமயம் மந்தம், எரிச்சல், வாந்தி, கழிச்சல் முதலியவைகளை உண்டாக்கும். உடம்பை ஊதச் செய்யும்.தூக்கத்தை கெடுக்கும். சாவை உண்டாக்கும்.

முறிவு:

சேரங்கொட்டை நெய் உடல் மேற்பட்டு உடல் ஊதிவிட்டால் செங்கல்லைப் பொடி செய்து உடலில் ஒற்றடம் போட்டு மெழுகையும் நல்லெண்ணையும் சேர்த்துக் காய்ச்சி மேலுக்குப் பூசினால் ஊதின உடல் வற்றிடும்.

சேராங்கொட்டைப் பாலால் உண்டாகும் புண்ணுக்கு முறிவு:

- கோரைக்கிழங்கு, சந்தனம், எள், ஏலரிசி, சரியெடை கூட்டி நீர் விட்டரைத்துத் தேனில் கலந்து உடல் மேல்பூச புண் நீங்கும்.
- சேங்கொட்டையை உட்கொண்டுவிட்டால் புளியிலையை நன்றாக இடித்து அதன் அளவிற்கு எட்டுபங்கு ஆற்றுநீர் சேர்த்து அதில் மேற்தோல் சீவின ஒரு இளநீரை போட்டு வேகவைத்து அத்தேங்காயின் வழுக்கையைத் தின்று அந்நீரையும் உட்கொள்ள சேங்கொட்டையின் வேகம் தனியும்.[8]

சேரும் மருந்துகள்:

- மகாவல்லாதி லேகியம்
- இரசகந்தி மெழுகு
- நந்தி மெழுகு
- சீன வல்லாதி மெழுகு
- நீரடிமுத்து வல்லாதி
- சரபராஜ குளிகை[32]

SEMECARPUS ANACARDIUM

Names in different languages:

English name	: Marking nut.
Tamil name	: Chenkottai.
Malayalam name	: Chermara.
Sanskrit name	: Bhallataka.
Telugu name	: Jeetivittulu.
Arabian name	: Beladin.
Gujarati name	: Bhiamu.
Persian name	: Biladur.
Panjabi name	: Bhela.
Marathi name	: Bibba.
Hindi name	: Bhela.

Habitat:

This tree is found growing on the sub Himalayan and tropical parts of india

Taxonomy of Semecarpus anacardium:

Kingdom	: Plantae
Subkingdom	: Tracheobionta.
Super division	: Spermatophyte.
Division	: Magnoliophyta.
Class	: Magndiopsida.
Subclass	: Rosidae.
Order	: Sapindales.
Family	: Anacardiaceae.
Genus	: Semecarpus.
Species	: Anacardiace.

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Parts used:

- Fruit (seed).
- Gum.
- Oil.

Description:

It is a moderate-sized deciduous tree found in the outer Himalayas and hotter parts of India up to 3500 ft. height. The plant is found in abundance in Assam, Bihar, Bengal and Orissa, Chittagong, central India and western peninsula of East Archipelago, Northern Australia.

It is a medium-to-large size tree, 15–25 m in height with grey bark exfoliating in small irregular flakes, leaves simple alternate, obviate – oblong, 30–60 cm long and 12–30 cm broad, rounded at the apex curvaceous glabrous above and more or less pubescent, beneath. The flowers are greenish white, in panicles and appear with new leaves in May and June, easily recognized by large leaves and the red blaze exuding resin, which blackens on exposure.

The nut is about 2.5 cm long, ovoid and smooth lustrous black. It is frequently found in drier rather than damp localities. The fruit ripens from December to March and are 2–3 cm broad. No specific soil affinity. It is a moderate shade bearer, obliquely ovoid or oblong drupe, 2.5 to 3.8 cm long, compressed, shining black when ripe, Seated on an orange-colored receptacle form of the disk, the base of the calyx and the extremity of the peduncle. The bark is grey in color and exudes an irritant secretion on incising[(33)].

Phenology:

Flowering season	:From May to August **.**
Fruiting season	: From August to February.
Seeding Season	: From August to February.
Leaf falling	: During hot season.

Phytochemistry:

The most significant components of the *S. anacardium.* Linn. are

- Bhilwanols
- Phenolic compounds
- Biflavonoids
- Sterols and Glycosides.

PHARMACOLOGY:

Anti atherogenic effect

The imbalance between the pro-oxidants and antioxidants is the main cause of development of atherosclerosis. To prevent such condition, antioxidant therapy is beneficial. *Semecarpus anacardium* (SA) shows such antioxidant property. It has capacity to scavenge the superoxide and hydroxyl radicals at low concentrations. The process of atherogenesis initiated by per oxidation of lipids in low-density lipoproteins was also found inhibited by semecarpus anacardium. Cardiac activity of SA, as it generally reduces the tissue and serum hyperlipidemia by the inhibition of intestinal cholesterol absorption coupled with peripheral disposal thus possessing anti-artherosclerotic activity[(34)].

It is possible that the beneficial anti atherogenic effect may be related to its antioxidant, anticoagulant, hypolipidemic, platelet anti-aggregation and lipoprotein lipase releasing properties. The mechanism of hypotriglyceridemic effect has also been shown to be partly due to stimulation of lipoprotein lipase activity.

Anti inflammatory activity

The anti inflammatory effects of SA nut extract on developing and developed adjuvant arthritis. *Semecarpus anacardium* significantly decreased the carrageen an-induced paw edema and cotton pellet granuloma. These results indicate the potent anti inflammatory effect and therapeutic efficacy of SA Nut extract against all phases of inflammation is comparable to that of indo methacin[(35)]

The ethyl acetate extract of SA led to the isolation of major active principle, tetra hydroamentoflavone (THA), a biflavonoid. The *in vitro* cyclooxygenase (COX-1)-catalyzed prostaglandin biosynthesis assay of THA gave an IC50 value of 29.5 μM (COX-1) and 40.5% inhibition at 100 g/mL (COX-2). The *in vivo* carrageenan-induced paw edema assay resulted in dose-dependent anti inflammatory effect of THA and the activity was comparable to that of ibuprofen[36].

The methanolic, ethanolic, chloroform, ethyl acetate and petroleum ether extracts of fruits of SA and tested to study the anti inflammatory activity using the technique of carrageenan-induced paw edema in albino rats. The extract showed significant anti inflammatory activity comparable to the reference standard aspirin[37]. The anti inflammatory activity of Semecarpus anacardium for both immunological and non immunological origin.The SA extract can inhibit pro inflammatory cytokine production[38].

Crude ethanolic extract of SA nuts was studied for its anti inflammatory activities *in vitro* using peripheral blood and synovial fluid mononuclear cells of healthy individuals and rheumatoid arthritis (RA) patients.

Semecarpus anacardium extract inhibited the spontaneous and LPS-induced production of pro inflammatory cytokines IL-1beta and IL-12p40 but had no effect on TNF-alpha and IL-6 production, both at protein and mRNA level. The crude extract also suppressed LPS-induced nuclear translocation of transcription factors, NF-kappa B and AP-1; the inhibition of NF-kappa B was through the inhibition of I kappa B α phosphorylation. The extract also suppressed LPS-activated nitric oxide production in mouse macrophage cell line, RAW 264.7[39].

For immune modulatory potency, anti oxidative, membrane stabilizing, tumors marker regulative, glucose level restoring and mineral regulation properties of nut extract in hepato cellular carcinoma and found to detoxify a potent Hepato carcinogen aflatoxin B_1 and causes its metabolites to excreted in urine[40].

In other case they explained the therapeutic effects of extract on the changes associated with collagen and glycosaminoglycan metabolism in adjuvant arthritic Wistar rats. Decreased levels of collagen and glycosaminoglycans (GAGS) components (chondroitin sulfate, heparan sulfate, hyaluronic acid) and increase in the levels of connective tissue degrading lysosomal glycohydrolases such as acid phosphatase, beta-glucuronidase, beta-*N*-acetyl glucosaminidase and cathepsin-D observed in arthritic animals were reverted back to near normal levels upon treatment with SA.

The nut milk extract modulates reactive oxygen/nitrogen species levels and anti oxidative system in adjuvant arthritic rats. A significant increase in the levels of lipid peroxides (LPOs), ROS (superoxide radical, hydroxyl radical, H_2O_2 and myeloperoxidase) and RNS (nitrate + nitrite) observed in adjuvant arthritic animals were found to be significantly decreased on administration of the drug at 150 mg/kg body weight/day.

Treatment with SA recouped the altered antioxidant defense components to near normal levels. These evidences suggest that the SA preparations are mainly used for irregularities caused during arthritis and to cure arthritis[41].

Kalpaamruthaa (KA), an indigenous-modified Siddha formulation, consists of SA nut milk extract and fresh dried powder of *Emblica officinalis* (EO) fruit along with honey. Kalpaamrutha was found to be nontoxic up to the dose level of 2000 mg/kg. Further, KA has been reported for its potent antioxidant analgesic, antipyretic and non-ulcerogenic properties. The anti inflammatory activity of SA in adjuvant-induced arthritic rat (AIA) model with reference to mediators of inflammation (lysosomal enzymes) and its effect on proteoglycans. The activities of various enzymes and levels of plasma protein bound carbohydrate components of glycoproteins were determined and were found to be elevated in arthritic rats when compared to control animals[42].

Antioxidant activity

Semecarpus anacardium has been reported in various studies to possess potent antioxidant activity. The antioxidant activity of the aqueous extract of nuts of

medicinal plant SA in AKR mouse liver during development of lymphoma. Administration of the aqueous extract of SA to lymphoma-transplanted mouse leads to increase in the activities of antioxidant enzymes, whereas LDH activity is brought down significantly indicating a decrease in carcinogenesis[43].

The antioxidant activity of ethyl acetate extract of stem bark of SA. Ethyl acetate extract showed the stronger antioxidant activity (due to presence of highest total phenolic content of 68.67% measured as pyrocatechol equivalent) compared to the other (hexane, chloroform and methanol) extracts.

The isolation of the ethyl acetate extract of SA stem bark yielded a bright-yellow solid crystal, which was identified as butein. This compound exhibited antioxidant activity (IC50 values of 43.28 ± 4.34 μg/ml), which was comparable to rutin, taken as a standard[44].

CNS activity

The beneficial effect of nuts of SA, extracted with milk, on CNS, mainly for its Locomotors and nootropic activities in different experimental animal models. The extract tested but a slight CNS depressant effect was noted with only 150 mg/kg of the extract and it was found to possess nootropic activity[45].

Antimicrobial activity

The aqueous and organic solvent extracts of the plant and screened for antimicrobial (disc diffusion method) and phytochemical properties. The petroleum ether (PEE) and aqueous extract fractions (AQE) showed inhibitory activity against *Staphylococcus aureus* (10 mm) and *Shigella flexneri* (16 mm) at 100 mg/ml, respectively. While chloroform extract showed inhibition against *Bacillus licheniformis*, *Vibrio cholerae* and *Pseudomonas aeruginosa*, the ethanol extract showed inhibition to *Pseudomonas aeruginosa* and *S. aureus*[46].

The alcoholic extract of dry nuts of SA (Bhallatak) showed bactericidal activity *in vitro* against three gram negative strains (*Escherichia coli, Salmonella typhi* and *Proteus vulgaris*) and two gram positive strains (*Staphylococcus aureus* and *Corynebacterium diphtheriae*). Subsequent studies have shown that the

alcoholic extracts of different parts of the plant (leaves, twigs and green fruit) also possess anti-bacterial properties, especially the leaf extract. No derma toxic effect (irritant property) was observed in the mouse skin irritant assay[47].

Hypoglycemic effect

The effect of ethanolic extract of dried nuts of SA on blood glucose and investigated in both normal (hypoglycemic) and streptozotocin-induced diabetic (antihyperglycemic) rats. The ethanolic extract of SA (100 mg/kg) reduced the blond glucose of normal rats The blood glucose levels were measured at 0, 1, 2 and 3 h after the treatment and antihyperglycemic activity of SA was compared with tolbutamide, a sulfonyl urea derivative used in diabetes mellitus[48].

Kalpaamruthaa (KA), a modified Siddha preparation, which contains Semecarpus anacardium studied for the variations in lipids, lipid-metabolizing enzymes and lipoproteins in cancerous animals and the effect of KA on the lipid metabolism. The increased levels of total cholesterol, free cholesterol, phospholipids, triglycerides and free fatty acids and decreased levels of ester cholesterol in plasma, liver and kidney found in cancer-suffering animals were reverted back to near normal levels on treatment with KA and SA. The effects of KA were found to be more effective than SA[49].

Anti-carcinogenic activity

Semecarpus anacardium nut extract for inhibitory effect on human breast cancer cells (T47D). Cytotoxicity analyses suggested that these cells had become apoptotic. *Semecarpus anacardium* was discovered to induce rapid Ca(2+) mobilization from intracellular stores of T47D cell line, and its Cytotoxicity against T47D was well correlated with altered mitochondrial transmembrane potential. At the molecular level, these changes are accompanied by decrease in Bcl(2) and increase in Bax, cytochrome c, caspases and PARP cleavage, and ultimately by internucleosomal DNA fragmentation.

Taken together, our results provide unprecedented evidence that SA triggers apoptotic signals in T47D cells[50].

The protective efficacy of preparation named as Kalpaamruthaa (KA) (includes SA nut milk extract, dried powder of Phyllanthus emblica fruit and honey) on the peroxidative damage and abnormal antioxidant levels in the hepatic mitochondrial fraction of 7,12-dimethylbenz(a) anthracene (DMBA)-induced mammary carcinoma rats. DMBA-treated rats also showed decline in the activities of mitochondrial enzymes. In contrast, rats treated with SA and KA showed normal lipid peroxidation antioxidant defenses in mitochondrial enzymes, and indicate the anticarcinogenic activity of KA during DMBA-initiated mammary carcinogenesis. On the basis of the observed results, KA can be considered as a readily accessible, promising and novel cancer chemopreventive agent[51].

Restoration of energy metabolism in leukemic mice treated by SA nut milk extract. Leukemia-bearing mice showed a significant increase in LPOs, glycolytic enzymes, a decrease in gluconeogenic enzymes and significant decrease in the activities of TCA cycle and respiratory chain enzymes as compared to control animals. *Semecarpus anacardium* treatment was compared with standard drug imatinib mesylate. *Semecarpus anacardium* administration to leukemic animals resulted in clearance of the leukemic cells from the bone marrow and internal organs[52]

Nephro toxicity:

The toxicity study on a few blood parameters in male albino rats at acute and sub-chronic levels with SA nut oil extract (50% w/v) in ground nut oil. Albino rats (Wistar strain) were treated orally with three sub-lethal doses. There was a significant decrease in hemoglobin percent and lowering of erythrocytes, indicating 'anemia' during toxicity study.

He also evaluated the acute and sub-chronic effect of crude extract on activity of some kidney enzymes GOT, GPT, SDH, LDH and histology of kidney of albino rat (Wistar strain) in either sex. Significant alteration in activity levels of marker enzymes of kidney as well as histological structure leading to nephritis were observed, indicating renal dysfunctioning in albino rat. Results exhibited nephrotoxicity inducing potential of SA nut oil extract[53].

சிற்றாமணக்கு

வேறு பெயர் : ஏரண்டம், சித்திரம், தலபோடம்

சுவை : கைப்பு

தன்மை : தட்பம்

பிரிவு : கார்ப்பு

செய்கை :

- பாற்பெருக்கி
- வாதமடக்கி

ஆமணக்கெண்ணெய்

குணம்:

"யேரண்டத்துநெய் யென்பது டற்கொடு
சீரண்டத்தணி செய்திடு நிசமே"

– தேரன் காப்பியம்

பொருள்:

ஆமணக்கு எண்ணெய், உண்மையாக உடலுக்கு மிகுந்த நன்மை கொடுக்கக் கூடியது.

"ஆமணக்கு நெய்யால் நலமுண்டாம் யாவர்க்கும்
பூமகணக்கு மேனி புரிகுழலே-வாய்மணக்க
கொள்ளில் வயிறுவிடுங் கோரமுள்ள வாயுவறும்
உள்ளில்வ்ரு குன்மம்போ மோது"

– அகத்தியர் குணபாடம்

சிறப்புப் பண்பு:

இது மருந்தின் வேகம், வலியினால் எருவாயிலுண்டாகும் அழலை நீக்கும்.

இதைப் பேதியாவதற்குக் கொடுக்கலாம். குழந்தைகளுக்கும் பிள்ளை பெற்ற பெண்களுக்கும் வயிறு கழிய கொடுப்பதற்கு இது சிறந்த மருந்தாகும்.

AAMANAKKU

Botanical name:		Ricinuscommunis
Family	:	Euphorbiaceae
Suvai	:	kaippu
Thanmai	:	Veppam
Pirivu	:	Kaarppu
Part used	:	Leaves, root and seed

Chemical constituents:

- Ricinine
- N-Dimethylricinine
- Asesquiterpenoid
- Ricinoleic acid
- Isoricinoleic acid
- Dyhydroxy stearic acid

Actions:

- Anti-vatha
- Immunomodulatory
- Hepatoprotective activity
- Anticancer activity

பனை வெல்லம்

குணம்:

"வட்டு பன வெல்லத்தால் மார்பெரிச்சல் குன்மமறும்
முட்டுந் திரிதோஷம் முன்னறிகா – கட்டுமடா
வாந்தி ருசியின்மை வாளா யுற்றிடினும்
சாந்தி பெருகுமென்றே சாற்று."

பனைவெல்லத்தால் சுரசன்னிபாதம், திரிதோஷதொந்தங்கள், அரோசகம், குன்மம், மார்புளரிச்சல், நீங்கும்.

உபயோகிக்கும் முறை:

பனை வெல்லத்தை கரைத்து வடிகட்டி பாகு எடுத்துப் பச்சை அரிசிமாவு கூட்டி அதிரசமாக சுட்டு உண்பதுண்டு. காபி, தேநீர் இவற்றில் சாதாரணமாக அஸ்கா, பூரா முதலியவற்றிற்கு பதிலாக இந்த பணைவெல்லத்தைப் போட்டு சாப்பிடுவதுண்டு. இதனால் தேகத்தின் வெப்பம் அடங்கும். பித்தம் தனியும். தேக ஆரோக்கியம் உண்டாகும்.[31]

சேரும் மருந்துகள்:

- நவரச மெழுகு
- நீரடிமுத்து வல்லாதி[32]
- மூசாம்பர மெழுகு[32]

Palm Jaggery

RESEARCH ARTICLES PUBLISHED IN JOURNALS:

1. Jaggery from Palmyrah palm (Borassus flabellifer L.)- Present status and scope Vengaiah PC, Ravindrababu D2, Murthy GN3 & Prasad KR4 Horticultural Research Station, Pandirimamidi-533288, Andhra Pradesh, College of Agricultural Engineering, Bapatla-522101, Andhra Pradesh Jaggery is a sugar rich product and medicine obtained by evaporation of sugarcane (Saccharum officinarum L.) juice or sap obtained from Palmyrah palm (Borassus flabellifer L.), Date palm (Phoenix dactylifera L.) or Coconut palm (Cocos nucifera L.). Among all Jaggery, palm Jaggery having its own importance. It usually contains 65-85% sucrose and 5-15% reducing sugars, and is consumed directly or used for preparation of sweet confectionary items and ayurvedic/traditional medicines, and it may have a role to reduce the chance of lung cancer. It is a good source of minerals like calcium, phosphorous and iron. Jaggery industry is one of the most important cottage level industries in India since ancient times and it is prepared mostly by small and marginal farmers. Besides India, countries like Pakistan, Bangladesh, Nepal, Burma and Philippines are also manufacturing Jaggery.[54]

2. Physicochemical and thermal properties of candy crystals prepared from palmyra-palm jaggery LEELA CHAUHAN1,2, KUMAR SATYA PRAKASH3, P.P. SRIVASTAV1 and KHALID BASHIR

Abstract

Candy crystals prepared from palmyra palm were analyzed for various physical and chemical properties during processing. The scanning electron microscopy displayed smooth and clear crystals of varied sizes. As the temperature of the jaggery increased from 28C to 108.4C, there was a subsequent increase in the total soluble solids from 28.8 to 848Brix. Thermal conductivity decreased from 0.44 to 0.14 W/mK, thermal diffusivity decreased from 0.31 3 1026 to 0.05 3 1026 m2/s and volumetric specific heat decreased from 1.35 3 103 to 0.64 3 103 kJ/m3_K. Thermal resistivity increased from 201.7 3 1022 to 755.1 3 1022 C_m/W. The maximum force taken as hardness was found to be 6,419 g. Power was found to be best for fitting the viscosity values (R250.93).[55]

பசுநெய்

குணம்:

"தாகமுழ லைசுட்கம் வாந்தி பித்தம் வாயுபிர
மேகம் வயிற்றெரிவு விக்கலழல்-மாகாசங்
குண்மம் வறட்சி குடற்புரட்ட லஸ்திசட்கஞ்
சொன்மூலம் போக்குநிறைத் துப்பு"

பொருள்:

பசுவின் நெய்யானது தாகம், உழலைப்பிணி, அதிசுட்கரோகம், வாந்தி, பித்தாதிக்கம், வாதவிஷம், வயிற்றெரிவு, பித்தவிக்கல், வயிற்றுவலி, குடல்நெளிதல், அஸ்திசூம்பல், மூலரோகம் ஆகியவற்றை நீக்கும்.[7]

4. MATERIALS AND METHODS

Selection of the test drug:

The test drug *Rathi nagara rasa mezhugu* was selected for the evaluation of toxicity studies in wister albino rats.

Ingredients of Rathi nagara rasa mezhugu:-

Sitramanakkennai (Castor oil)	-	¼ palam (8.75 gm)
Gandhakam (Sulphur)	-	1 palam (35 gm)
Serankottai *(Semecarpus anacardium*)	-	30 Nos
Vaalai rasam (Mercury)	-	1 palam (35 gm)
Pasu nei (Ghee)	-	1 palam (35 gm)
Panai vellam (Palm jaggery)	-	2 palam (35 gm)

Procurement of the raw drugs:

Rasam (Mercury), Ganthagam (Sulphur), Serankottai (*Semecarpus anacardium*) was procured from a reputed country shop, Broadway, Chennai. The following ingredients are Sitramanakku ennai (castor oil), Pasu nei (ghee), Panai vellam (palm jaggery) from procured from Tambaram market, Chennai .

Identification and authentication of the raw drug:

The herbal drug were identified and authenticated by Botanist, NIS Tambaram sanatorium Chennai (Certificate no NISMB2942017). The metal drugs were identified by pharmacologist, dept. of Gunapadam, NIS, Tambaram sanatorium, Chennai. (Certificate no NIS/Gunapadam/Au2017/18).

The purification process:

Purification of Rasam (Mercury):

35mg of Mercury triturated with brick powder and turmeric powder for one hour respectively, and washed with water. Then the mercury was boiled with 1 lit of the juice of *Kuppai meni* (Acalypha indica) until the juice evaporated completely.

Purification of Gandhagam (Sulphur):

Sulphur was placed in an iron spoon. A small quantity of cow's butter was added and the spoon was heated till the sulphur melts, then the melted sulphur immersed in the milk. This process was repeated for 21 times.

Purification of Serankottai:(Semecarpus anacardium):

Step 1:

The nose of the Serankottai should be chopped initially and put into the lime stone.

Step 2:

The Serankottai should be boiled with Puliyilai kudineer (Tamarindus indicus leaf extract) Purasampattai kudineer,(Butea monospermea), Pasum saanappaal (cow dung extract), Sottrukatralai (Aloe barbadensis) juices respectively and then wash with water dried.

4.1 PREPARATION:

Step 1:

First castor oil is taken in a vessel and heated. Then Purified sulphur is powdered and mixed with the heating castor oil. When the sulphur melts, Semecarpus seeds are cut into two pieces and put in the oil. Then thailam is taken when semecarpus seeds turn red and floats. The thailam is called ***Rathi nagara thailam***.

S**tep 2:**

Mercury and Rathi nagara thailam is mixed and grinded. When mercury is grinded well, Ghee and palm jaggery is added and grinded to get *Rathi nagara rasa mezhugu.*

THERAPEUTIC DOSAGE:

3 to 4 kundri (490-520 mg), twice a day

Duration of treatment is 3days

VEHICLE:

Palm jaggery

THERAPEUTIC USES:

- Linga puttru (Penial cancer),
- Algul puttru (Cervix cancer),
- Araiyaappu (Adenitis),
- Kandamaalai(Cervical adenitis),
- Karunkuttam, Senkuttam (Leprosy),
- Megaranam (Syphilis),
- Kaalkai mudakku (Rheumatoid Arthritis).

Mercury Before Purification

Mercury After Purification

Sulphur Before Purification

Sulphur After Purification

Serankottai Before Purification

Serankottai After Purification

STANDARDIZATION OF RATHI NAGARA RASA MEZHUGU

4.2 QUALITATIVE ANALYSIS

PHYSICO-CHEMICAL ANALYSIS OF RATHI NAGARA RASA MEZHUGU (RNM):

The physico- chemical properties of Rathi nagara rasa mezhugu is carried as per standard procedure at The Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai.

1. Moisture Content:

An accurately weighed 1g of *Rathi nagara rasa mezhugu* formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0 C in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.

2. Determination of total ash:

Weighed accurately 1g of *Rathi nagara rasa mezhugu* formulation was added in crucible at a temperature 600^0 C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

3. Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

4. Determination of water soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0 C in a muffle furnace. Difference in weight of ash and weight of water.[57]

5. Determination of water soluble Extractive:

1gm of air dried drug, coarsely powered Rathi nagara rasa mezhugu was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 1000 C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs. [57]

6. Determination of alcohol soluble extractive:

1 gm. of air dried drugs, coarsely powdered Rathi nagara rasa mezhugu was macerated with 100 ml. alcohol in closed flask for 24 hrs. With frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 25ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 1000C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.[57]

THE PRELIMINARY PHYTOCHEMICAL SCREENING TEST:

The preliminary phytochemical screening test was carried out for each extracts of ***Rathi nagara rasa mezhugu*** as per the standard procedure at The Tamilnadu DR. M.G.R. Medical University, Guindy, Chennai-32.

Detection of alkaloids:

Extracts were dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test:

2 ml of extract was treated with few drops of Mayers' reagent; formation of yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test:

2 ml of filtrate was treated with Wagner's reagent. Formation of brown /reddish precipitate indicates the presence of alkaloids.

Detection of carbohydrate:

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for presence of carbohydrates.

Molisch's test:

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of carbohydrates.

Benedict's test:

Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of saponins:

Froth test:

Extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 centimeter layer of foam indicates the presence of Saponins.

Foam test:

0.5-gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of saponins.

Detection of phytosterols

Salkowski's test:

Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Ferric Chloride test:

2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test:

To the extracts, 1% of gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline reagent test

Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow colour then on addition of diluted hydrochloric acid it becomes colourless, it indicates the presents of flavonoids.

Lead acetate test

Extract was treated with few drops of lead acetate solution, yellow colour precipitate indicates presence of flavonoids.

Detection of diterpenes;

Copper Acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper Acetate solution, formation of emerald green colour indicates the presence of diterpenes.

Test for gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

Detection of Glycosides

Liebermann's test

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet colour change into blue and green indicates presence of Glycosides.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

Result

The Preliminary phytochemical studies of extract of Rathi naga rasa mezhugu in various solvents were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of Rathi nagara rasa mezhugu.

BIO -CHEMICAL ANALYSIS OF RATHIRA NAGARASA MEZHUGU (RNM):

The bio–chemical analysis of Rathi nagara rasa mezhugu as done at Biochemistry lab, National Institute of Siddha, Chennai, 47

Preparation of Extract:

5gm of ***Rathi nagara rasa mezhugu(RNM)*** is weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Dark brown in colour	
2.	**Test for Silicate:** a. A little (500mg) of the sample is shaken well with distilled water. b. A little (500mg) of the sample is shaken well with con. HCl/Con. H_2So_4	Sparingly soluble completely soluble	Presence of Silicate
3.	**Action of Heat:** A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.	White fumes evolved	Presence of Carbonate
4.	**Flame Test:** A small amount (500mg) of the sample is made into a paste with con. HCL in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame appeared.	Absence of Copper
5.	**Ash Test:** A filter paper is soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited	No yellow colour flame appeared	Absence of sodium

Test for Acid Radicals

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Sulphate:** 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No cloudy appearance present	Absence of Sulphate
	Test For Chloride: 2ml of the above prepared extracts is added with 2ml of dil-HCl is added until the effervescence ceases off...	No cloudy appearance present	Absence of Chloride
3.	**Test For Phosphate:** 2ml of the extract is treated with 2ml of dil.ammoniummolybdate solution and 2ml of con.HNO_3	No yellow precipitate present	Absence of Phosphate
4.	**Test For Carbonate:** 2ml of the extract is treated with 2ml dil. magnesium sulphate solution	cloudy appearance present	Presence of carbonate
5.	**Test For Nitrate:** 1gm of the substance is heated with copper turning and concentrated H_2SO_4 and viewed the test tube vertically down.	No brown gas is evolved	Abesence of Nitrate
6.	**Test For Sulphide:** 1gm of the substance is treated with 2ml of con. HCL	No rotten Egg Smelling gas is evolved	Absence of Sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract is added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	Absence of Cloudy appearance	Absence of fluoride and oxalate

8.	**Test For Nitrite:** 3drops of the extract is placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes	Absence of Nitrite
9.	**Test For Borate:** 2 Pinches (50mg) of the substance is made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No bluish green colour flame appeared	Absence of borate

II.Test for Basic Radicals

1.	**Test For Lead:** 2ml of the extract is added with 2ml of dil.potassium iodine solution.	No yellow Precipitate is obtained.	Absence of Lead
2.	**Test For Copper:** One pinch (50mg) of substance is made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.	No blue colour precipitate is formed.	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide is added in 5 drops to excess.	yellow colour appearance	Presence of aluminum
4.	**Test For Iron:** To the 2ml of extract add 2ml of dil.ammonium solution To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Mild red colour appear Blood red colour appearance	Iron present

5.	**Test For Zinc:** To 2ml of the extract dil.sodium hydroxide solution is added in 5 drops to excess and dil.ammonium chloride is added.	white precipitate is formed	presence of Zinc
6.	**Test For Calcium:** 2ml of the extract is added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance or white precipitate formation is present	Presence of calcium
7.	**Test For Magnesium:** To 2ml of extract dil.sodium hydroxide solution is added in drops toexcess.	white precipitate is obtained	presence of Magnesium
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added.	No brown colour is appeared	Absence of ammonium
9.	**Test For Potassium:** A pinch (25mg) of substance is treated of with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	Yellowish precipitate is obtained.	Presence of Potassium
10.	**Test For Sodium**: Pinches (50mg) of the substance is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared	Absence of sodium

11.	**Test For Mercury**: 2ml of the extract is treated with 2ml of dil.sodium hydroxide solution.	yellow precipitate is obtained	presence of mercury
12.	**Test For Arsenic:** 2ml of the extract is treated with 2ml of dil.sodium hydroxide solution.	No brownish red precipitate is obtained	Absence of arsenic

Test for other constituents

1.	**Test For Starch :** 2ml of extract is treated with weak dil.iodine solution	No blue colour formation	Absence of starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No brick red colour developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the extract is treated with 2ml of dil.potassium lodide solution. b) 2ml of the extract is treated with 2ml of dil.picric acid.	reddish brown precipitation appears yellow precipitation appears	Presence of Alkaloid
4.	**Test For Tannic Acid:** 2ml of extract is treated with 2ml of dil.ferric chloride solution	No Black precipitate is obtained	Absence of Tannic acid
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.	Potassium permanganate is not decolourised	Absence of unsaturated compound
6.	**Test For Amino Acid:** 2 drops of the extract is placed on a filter paper and dried well. 20ml of Biurette reagent is added**.**	Violet colour is not developed	Absence of amino acids
7.	**Test For Type Of Compound:** 2ml of the extract is treated with 2 ml of dil.ferric chloride solution.	No specific colour formation	Phenols absent

4.3. QUANTITATIVE ANALYSIS

4.3.1 HEAVY METAL ANALYSIS

The analysis of heavy metals and trace elements were estimated by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

ICP-OES:

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in (table 5), the wavelength of analytical lines is given in table (5) and the test drug *Rathi nagara rasa mezhugu* underwent microwave digestion for sample preparation.

Table 4.2.3 : ICP- OES Operating Conditions

RF frequency	40M Hz
Range	165-782 mm
Detection limit	Up to ppm level using SCD detector

4.3.2 ANALYSIS OF PARTICAL SIZE

The particle size of the *Rathi nagara rasa mezhugu* was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

HR SEM:

The SEM analysis is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

Resolution : 1.2 nm gold particle separation on a carbon substrate

Magnification : From a min of 12 X to greater than 1,00,000 X.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micron in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particle was calculated.

4.4 TOXICITY STUDIES OF RATHI NAGARA RASA MEZHUGU

To evaluate the Preclinical safety profile of *Rathi nagara rasa mezhugu* acute and sub acute toxicity study carried out as followed

Principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of animals and the study design. Institutional Animal Ethical Committee approval number: (IAEC). NIS/IAEC-II/14/2016 for acute toxicity study and repeated dose 28-day oral toxicity study.

1. ACUTE TOXICITY STUDY OF RATHI NAGARA RASA MEZHUGU[58]

Experimental Animals:

Species	:	Wister Albino Rats
Sex	:	Female
Age/weight	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages with bedding with Husk
Husbandry	:	12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water ad libitum
Identification	:	Animals will be kept in Polypropylene cages and numbered

Experimentation Details of Acute Toxicity Study:

Groups/Treatment regimen	:	Grouped by randomization
Test Guideline	:	OECD-423
Length of exposure to test substance	:	Once single dose
No of Animals	:	3 Female/ group
Control group	:	Vehicle (palm jaggery)
Test groups	:	*Rathi nagara rasa mezhugu* 50, 2000 mg/kg. b.wt

The Female Albino Rats of weighing 150-200g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute of Siddha, Chennai. Animals were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet (Sai Meera Foods Pvt. Ltd., Bangalore). The animals were treated with *Rathi nagara rasa mezhugu* by oral route for one day and monitored for behavioral parameters for the first 4 hours (1/2 hr, 1hr,2 hr, 3 hr,4 hr) after drug administration. Body weight of the animal will be monitored at weekly intervals. All animals will be weighed and sacrificed under the injection of Pentothal Sodium on the 15th day of the Study period. The toxicological effect was assessed on the basis of mortality.

Preparation of Test Drug Doses:

Groups	Dose	No. of Rat
Group I	Vehicle control (palm jaggery)	3 Female
Group II	50 mg/kg b.wt	3 Female
Group III	2000 mg/kg b.wt	3 Female

Route of administration

Oral route was selected because it is the normal route of clinical administration.

Administration of Dose

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each step. A single dose of the solution (50, 2000mg/kg) was consecutively administered by oral gavages using intubation cannula. The food was withheld for another 4hrs after dosing and administration of the drug. As per the guideline, the starting dose level was taken as 50mg/kg body weight.

Observations:

Observations were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- ✓ ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation
- ✓ All rats were observed twice daily for 14 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation:

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, pilo erection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality

Gross necropsy:

At the end of the 14 th day, all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, lungs, heart, spleen, liver, kidneys, ovary and uterus of all animals also were grossly examined.

2. REPEATED DOSE 28-DAY ORAL TOXICITY STUDY OF RATHI NAGARA RASA MEZHUGU[59]

Experimental Animals:

Species	:	Wister Albino Rats
Sex	:	Male and Female
Age/weight at start of test	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages with bedding with Husk
Husbandry	:	12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water ad libitum
Identification	:	Animals will be kept in Polypropylene cages and numbered

Experimentation Details of Repeated dose 28 days Toxicity Study:

Groups/Treatment regimen	:	Grouped by randomisation
Test Guideline	:	OECD-407
Length of exposure to test substance	:	28 days
No of Animals	:	3 Female+3 Male / group
Control group	:	Vehicle (palm jaggery Solution)
Test groups	:	*Rathi nagara rasa mezhugu* (Low dose, Mid dose, High dose)

The 24 Wister albino rats of both sexes selected randomly. The animals were divided into four groups. Each group consists of 6 animals. The first group treated as vehicle control and second, third and fourth group were treated with *Rathi nagara rasa mezhugu* Low-dose (46.8mg), Mid dose (234mg), and High-dose (468 mg) respectively. The control animals were administered with palm jaggery as a vehicle. The other animals treated with *Rathi nagara rasa mezhugu* which was mixed with palm jaggery at the dose levels of low dose 46.8mg/ kg b.wt, Mid dose234 mg/kg b.wt and High dose 468 mg/kg b.wt. For 28 days. The administration was given by oral, once daily for 28 consecutive days. The animals were observed the behavioral parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Food & water intake were Calculated daily. All the animals were sacrificed at the end of the study (29 days) by using the injection of Pentothal Sodium. Blood was collected from the anesthetized animals from the abdominal aorta for the following investigations like Hematology, Biochemical analysis. Gross pathological changes were monitored then the organs were studied by histo pathological examination.

The doses (Low, Mid, High dose) were fixed from the result from the acute toxicity study

Group	Dose	No. of Rats
Group I	Vehicle control (palm jiggery)	6 (3M + 3F)
GroupII	Low dose (RNM 46.8 mg/Kg b.wt)	6 (3M+ 3F)
GroupIII	Mid dose (RNM 234 mg/Kg b.wt)	6 (3M+ 3F)
GroupIV	High dose (RNM 468 mg/Kg b.wt)	6 (3M + 3F)

Observations:

Experimental animals were kept under observation throughout the course of study for the following

- ✓ All rats were observed twice daily for 28 days
- ✓ Body weight were Calculated weekly once
- ✓ Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Laboratory Investigations:

On the 29th day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 29th day and Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, lungs, heart, spleen, liver, kidneys, testes, ovary and uterus of all animals also were grossly examined.

Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs, testes, ovary and uterus and stomach of the animals were preserved, and they were subjected to histopathological examination.

Histopathological investigation of the vital organs was done. The organ pieces (35µm thick) of all the animals (low, mid, high) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50^0 C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, and hematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D Graph Pad Instat-3

RESULTS

After the preparation of the test drug Rathi nagara rasa mezhugu it undergone Biochemical, Physico chemical , ICP-OES, HR-SEM Analysis and Toxicity study and their results are given below.

Results of Analytical studies on Rathi nagara rasa mezhugu:

Table-1: Physico-chemical properties of Rathi nagara rasa mezhugu (RNM)

S.No	Parameters	Percentage
1	Loss on drying	1.98%
2	Total ash value	2.09%
3	Acid insoluble ash	Less than 1%
4	Water insoluble ash	1.45%
5	Water soluble extraction	3.77%
6	Alcohol soluble extraction	15.59%

Table: 2 Colour and nature of Rathi nagara rasa mezhugu (RNM)

S.no	Parameters	Results
1	Appearance	Dark brown coloured semisolid substance
2	PH at 25 c (1%w/v solution)	3.65
3	Solubility	Partially soluble in water Partially soluble in acid Dispersed in alcohol

Table 3: Biochemical analysis of Rathi nagara rasa mezhugu

S.NO	PROCEDURES	RESULTS
1	Test for Ammonium	-ve
2	Test for Sodium	-ve
3	Test for Magnesium	+ve
4	Test for Aluminum	+ve
5	Test for Potassium	+ve
6	Test for Calcium	+ve
7	Test for Ferrous Iron	+ve
8	Test for Zinc	+ve
9	Test for Arsenic	-ve
10	Test for Mercury	+ve
11	Test for Lead	-ve
12	Test for Sulphate	-ve
13	Test for Chloride	-ve
14	Test for Phosphate	-ve
15	Test for Carbonate	+ve
16	Test for Fluoride & Oxalate	-ve
17	Test for Starch	-ve
18	Test for Reducing sugar	-ve
19	Test for Alkaloids	+ve
20	Test for Amino Acids	-ve
21	Test for Unsaturated compounds	-ve

(+) – present; (-) - Absent

The biochemical analysis show the presence of carbonate, aluminum, iron, zinc, calcium, magnesium, potassium, mercury, alkaloid in Rathi nagara rasa mezhugu

Table 4: Phytochemical Analysis for Rathi nagara rasa mezhugu

S.no	Phyto chemicals	Test name	H 2O EXTRACT
1	Alkaloids	Mayer's test	-ve
		Wagner's test	-ve
2	Carbohydrates	Molisch' test	-ve
		Benedict's test	-ve
3	Glycosides	Libermann burchard's test	+ve
4	Saponins	Froth test	-ve
		Foam test	-ve
5	Phytosterols	Salkowski's test	-ve
6	Phenols	Ferric choloride test	-ve
7	Tannins	Gelatin test	-ve
8	Flavonoids	Alkaline reagent test	-ve
		Lead acetate test	-ve
9	Proteins and amino acids	Xanthoproteic test	-ve
10	Diterpenes	Copper acetate test	-ve
11	Fixed oil and fat	Spot test	+ve
12	Quinone	NAOH+ extract	-ve

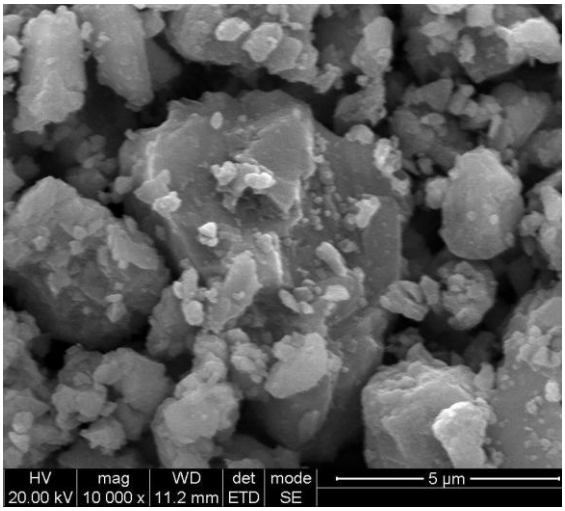
The phytochemical analysis shows the presence of Glycosides and fixed oil and fat.

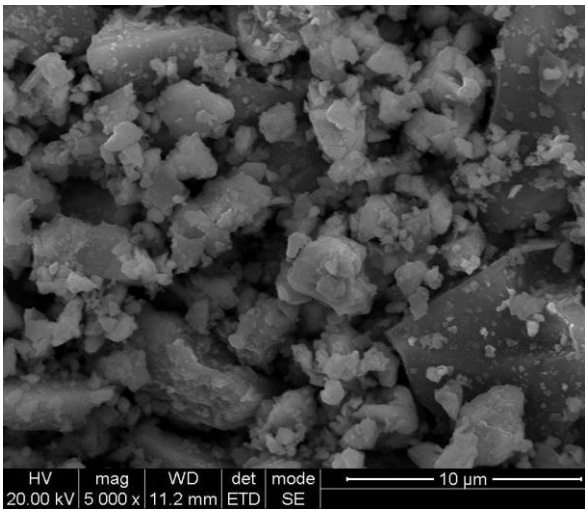
Table : 5 (ICP-OES) of Rathi nagara rasa mezhugu:

S.no	Elements	Wavelength in nm	Rathi nagara rasa mezhugu mg/L
1	Aluminium	Al 396.152	BDL
2	Arsenic	As 188.979	BDL
3	Calcium	Ca 315.807	35.160
4	Cadmium	Cd 228.802	BDL
5	Copper	Cu 327.393	BDL
6	Iron	Fe238.204	10.243
7	Sodium	Na 589.592	14.890
8	Zinc	Zn 213.910	380.247
9	Lead	Pb 220.353	BDL
10	Magnesium	Mg 285.213	BDL
11	Mercury	Hg 253.652	03.214
12	Potassium	K 766.491	05.807
13	Phosphorus	P 213.617	126.321
14	Nickel	Ni 231.604	BDL

(ICP-OES) results showed that the Heavy metals like Aluminum, Arsenic, Copper, Lead, Magnesium, and Nickel were found below detection level. Mercury (03.214 mg/L) was found within the permissible level in RNM. It also shows the presence of physiologically important minerals like sodium, potassium, iron, zinc, Calcium, phosphorus.

Image :1 HR- SEM Analysis - Determination of particle size of Rathi nagara rasa mezhugu:





Results and Interpretation of SEM analysis:

The morphology of the Rathi nagara rasa mezhugu (RNM) sample can be determined by SEM (FEI Quanta). We have observed from SEM photographs that particles are spherical in shapes and sizes are in the range from 0.5 micron to 2 microns. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. Rathi nagara rasa mezhugu exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation.

ACUTE TOXICITY STUDY

Table6. Behavioral Signs of Acute Toxicity Study of Rathi nagara rasa mezhugu (RNM) [58]

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	50	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3	2000	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2.Aggressiveness 3.pilo erection 4.Grooming 5.Gripping 6.Touch Response 7. Motor Activity 8. Tremors 9.Convulsions 10.Muscle Spasm 11.Catatonia 12. Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20.Mortality

\+ Presence of Activity - Absence of Activity

All the data were summarized in the form of (table-6) revealed that there was no abnormal signs and behavioral changes in all animals at the dose level of 50, 2000 mg/kg body weight administered orally, during the study period.

There was no mortality observed after dosing of Rathi nagara rasa mezhugu (RNM) up to 2000mg/kg body weight during the study period of 14 days. This indicates that the LD50 of Rathi nagara rasa mezhugu is more than 2000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group.

At the end of the 14 the day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

28 Day Repeated Dose Oral Toxicity Study

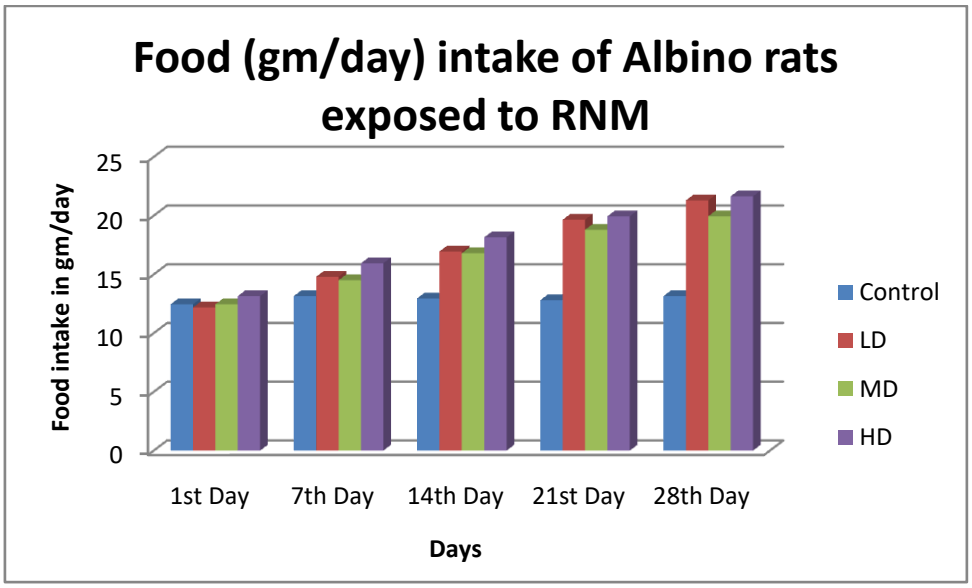
Food intake of the test group animals were observed and compared with control group during the study period. (Table 7), Food consumption of the animals is significantly differed but they are within physiological limit.

Table: 7 Food (g/day) intakes of albino rats exposed to RNM

Dose (mg/kg/ day)	1st day	7th day	14th day	21st day	28th day
Control	12.45±0.16	13.15±0.16	12.95±0.21	12.8±0.21	13.15±0.16
Low dose	12.8±0.21**	14.8±0.21**	16.95±0.38**	19.65±0.38**	21.3±0.32**
Mid dose	12.45±0.54**	14.5±0.54**	16.8 ±0.54**	18.8±0.54**	19.95±0.71**
High dose	13.15±0.16**	15.95±0.71**	18.15±1.25**	19.95±0.71**	21.65±0.71**

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 3:



28 Day Repeated Dose Oral Toxicity Study

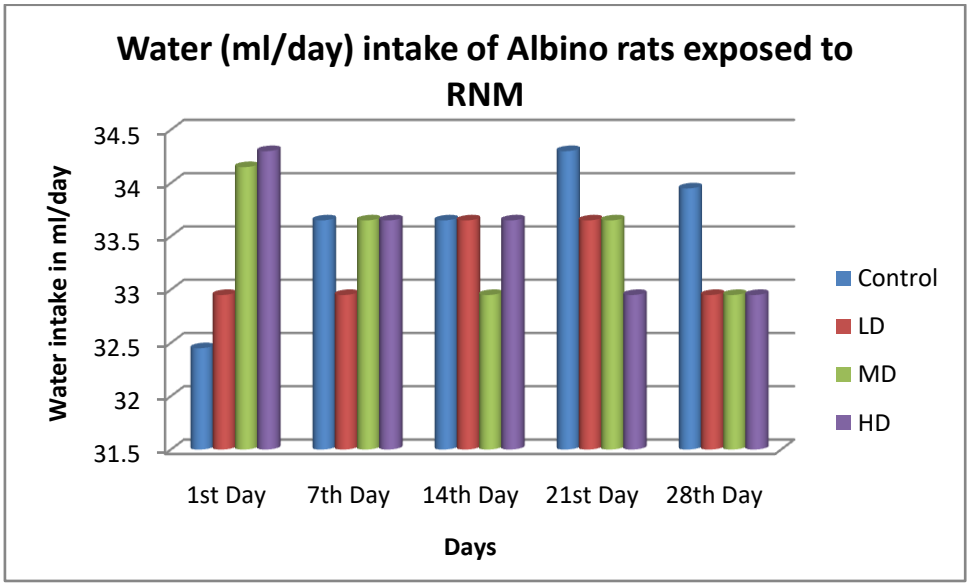
Water intake of control and test group of animals observed during the study period. (Table 8), There was significant difference occurs in the group low and mid at 28 days compared with control group.

Table:8 Water (ml/day) intake of albino rats exposed to RNM

Dose (mg/kg/day)	1st day	7th day	14th day	21st day	28th day
Control	32.45±0.43	33.65±0.38	33.65±0.38	34.3±0.32	33.95±0.71
Low dose	32.95±0.38*	32.95±0.38*	33.65±0.38	33.65±0.38*	32.95±0.38**
Mid dose	34.15±0.93*	33.65±0.38	32.95±0.38*	33.65±0.38*	32.95±0.38**
High dose	34.3±1.86	33.65±0.38	33.65±0.38	32.95±0.38	32.95±0.38

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 4:



28 Day Repeated Dose Oral Toxicity Study

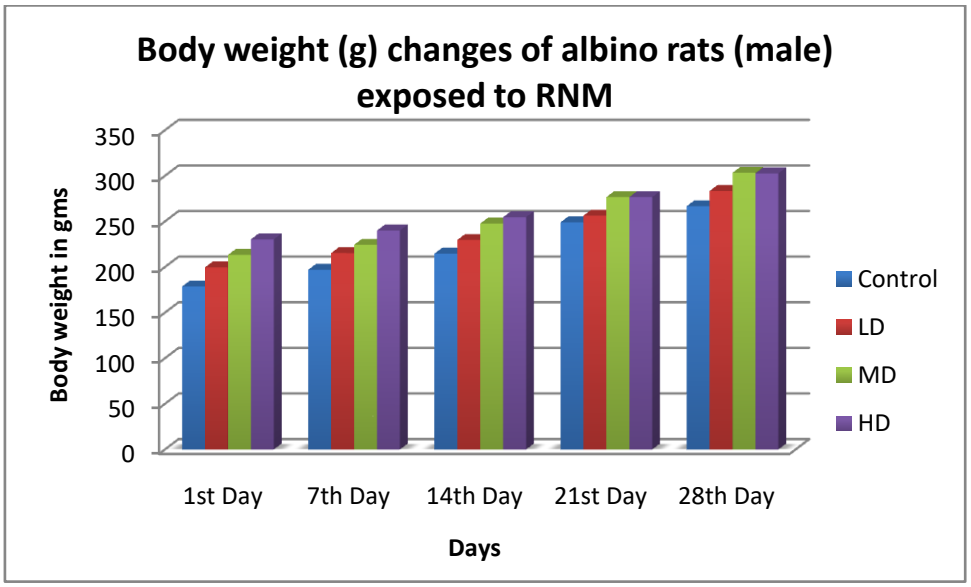
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 9) There was significant difference occurs in the group mid and high dose at 28 days compared with control group.

Table: 9 Body weight (g) changes of albino rats (male) exposed to RNM

Dose (mg/kg/day)	1st day	7th day	14th day	21st day	28th day
Control	178.66±19.03	197 ±27.87	214.66±29.73	249±37.51	266.66±4.52
Low dose	199.66 ±9.07	215.33±5.50	229.66±10.59	256.3±6.35	283.33±15.27
Mid dose	213.33 ±5.50**	224.33± 6.02*	247.66±12.09	276.66±7.63*	303.33±7.63*
High dose	230.33±21.59**	240 ±20**	254.66±22.81**	276.66±27.53	302.66±37.16*

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 5:



28 Day Repeated Dose Oral Toxicity Study

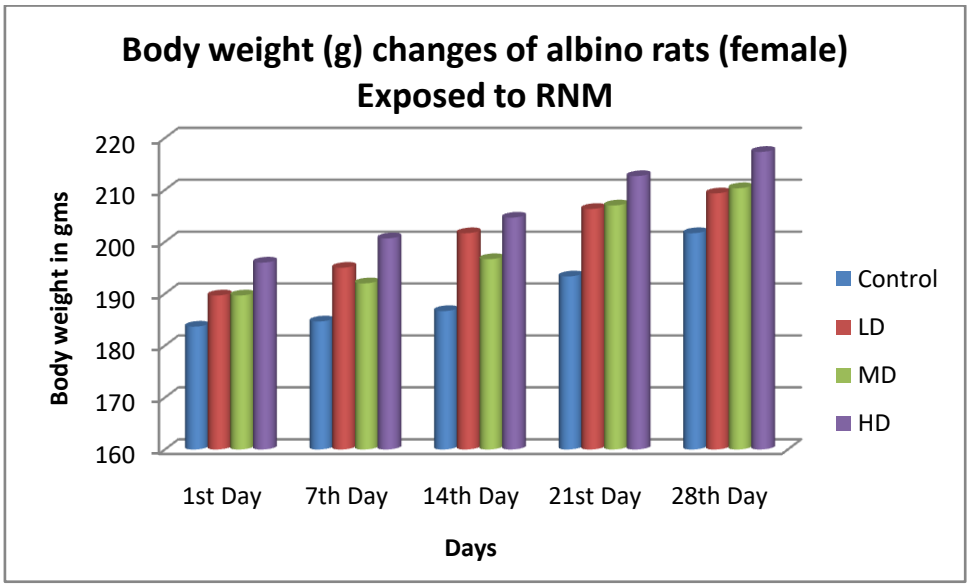
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 10) There was significant difference occurs in all test groups 28 days compared with control group.

Table: 10 Body weight (g) changes of albino rats (female) exposed to RNM

Dose (mg/kg/day)	1st day	7th day	14th day	21st day	28th day
Control	183.66±5.50	184.66±4.50	186.66±2.51	193.33±3.05	201.66±2.08
Low dose	189.66±8.50	195±3*	201.66±6.65**	206.33±6.65**	209.33±6.65
Mid dose	189.66±12.09	192±11.13	196.66±10.06*	207±2.64**	210.33±2.51
High dose	196±7	200.66±5.03**	204.66±3.51**	212.66±6.42**	217.33±11.01**

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 6:



28 Day Repeated Dose Oral Toxicity Study

The results of the **Hematological investigations** conducted at the end of the study, the groups revealed slightly significant changes in levels of hematological parameters, when compared with control group hematological parameters towards normal, when compared with control group. (Table 11)

Table: 11 Effect of RNM on Hematological Parameters

Parameter	control	LD	MD	HD
RBC ($x10^6$µl)	6.68±0.40	7±0.35	6.03±0.52	6.13±0.72
WBC($x10^3$µl)	8.3±2.02	7.53±1.92	9.58±1.27	9.08±3.32
Platelets ($x10^3$µl)	558.5±74.75	728.33±108.56	684.33±180.21	743±157.30
HGB(g/dl)	11.61±1.50	14.41±2.08*	11.81±1.72	12.53±1.94
Neutrophil (10^3mm^3)	1.4±0.09	1.33±0.16	1.78±0.34	1.91±0.47*
Lymphocyte (%)	88.48±3.96	69.3±1.44	69.05±4.21**	75.2±6.43**
Monocyte(%)	4.41±0.72	3.78±1.53	3.3±0.51	4.53±0.30
Eosinophil(%)	1.31±0.22	1.66±0.13**	1.08±0.16	1.25±0.16
Basophil(%)	0.16±0.40	0.33±0.51	0.16±0.40	0.16±0.40.
MCH(pg)	18.05±4.51	17.58±0.59	21.53±0.86*	22.06±0.90*
MCV(fl)	58.28±3.30	58.86±5.24	58.35±4.21	55.55±4.75

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 7:

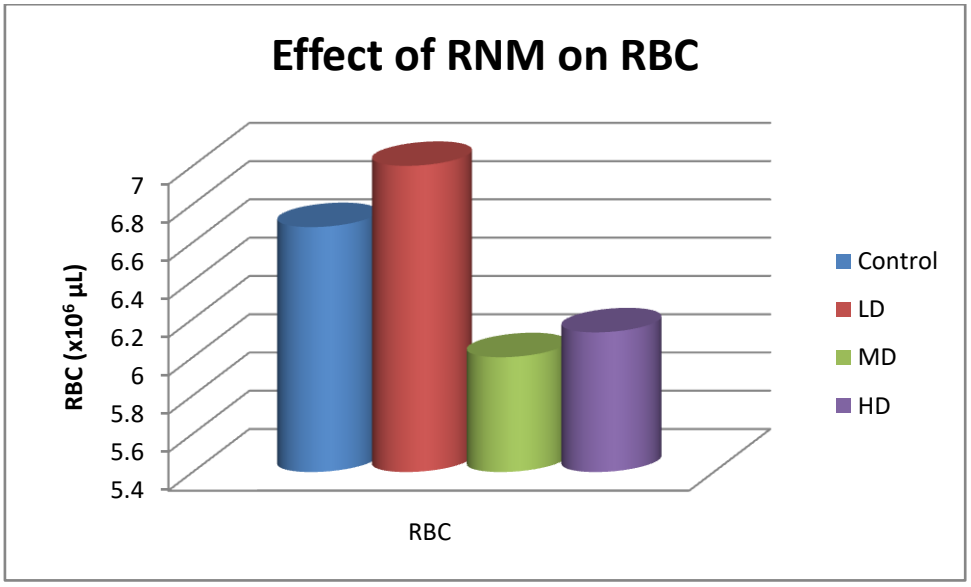


Figure 8:

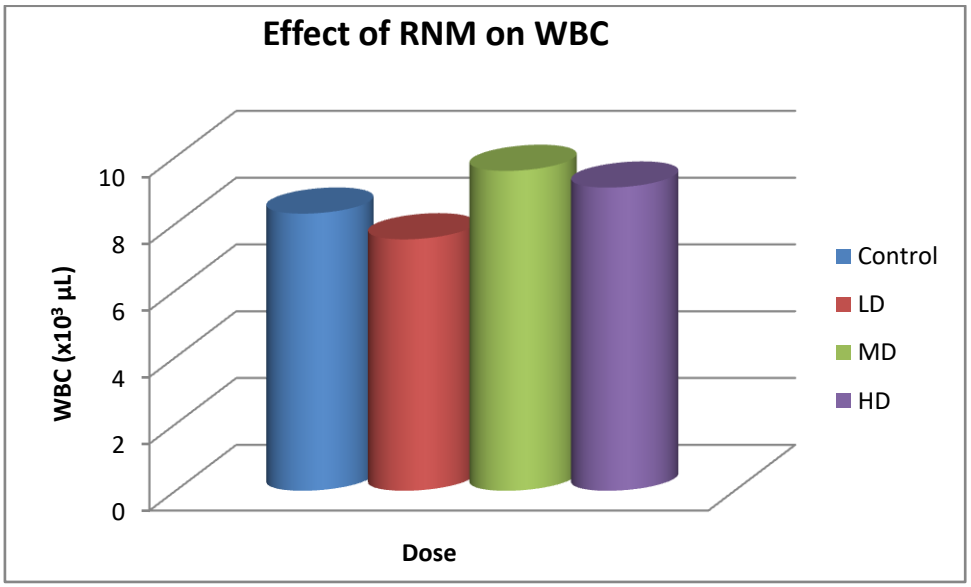


Figure 9:

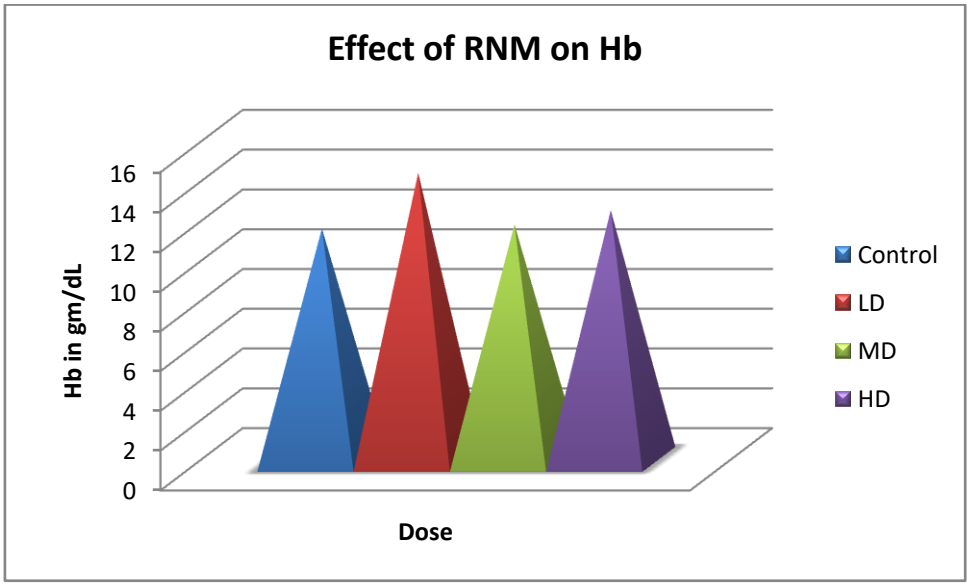


Figure 10:

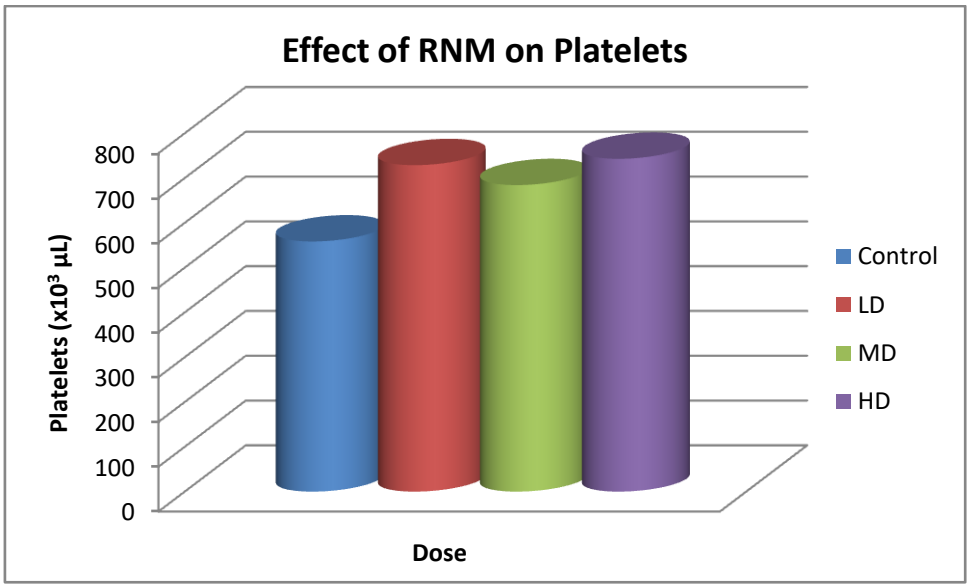


Figure 11:

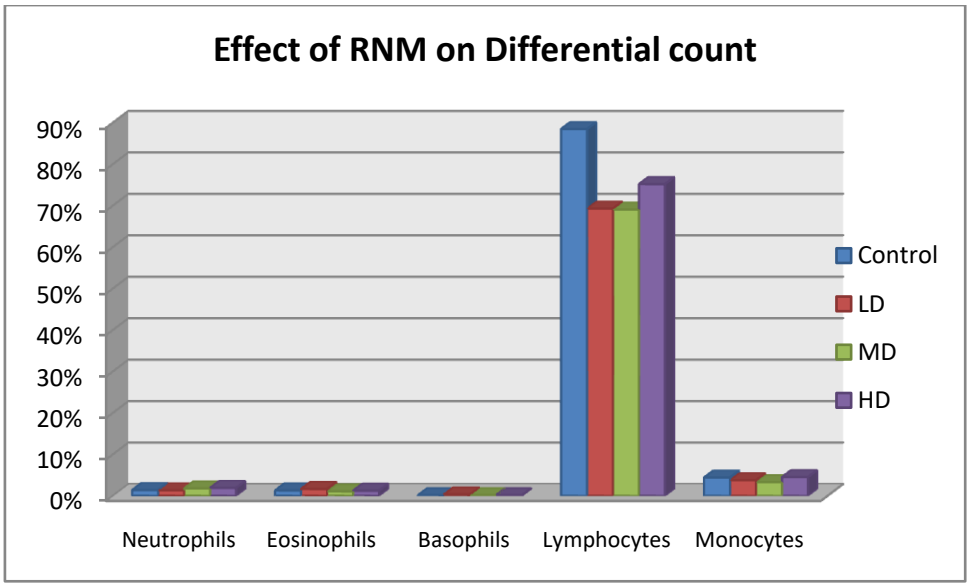
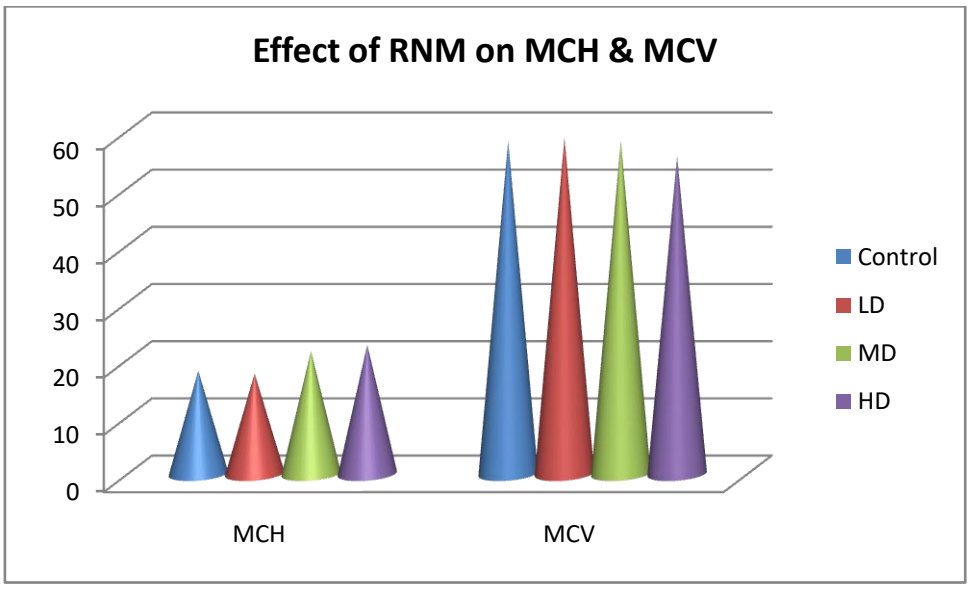


Figure 12:



28 Day Repeated Dose Oral Toxicity Study

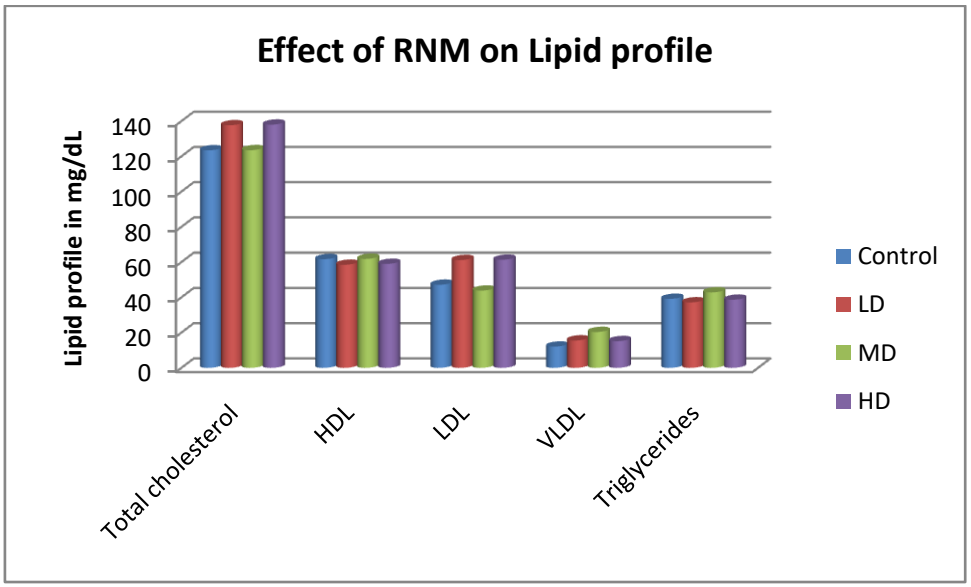
Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups there was significant changes present in VLDL Mid dose, when compared with the control group. At the values were normal biological limits. (Table 12)

Table12 Effect of Rathi nagara rasa mezhugu on Biochemical parameters

Dose (mg/kg)	control	LD	MD	HD
Total cholesterol (mg/dl)	123.45±14.01	137.67±18.37	123.53±13.98	138.03±26.14
HDL(mg/dl)	61.67±7.58	58.5±4.08	61.83±8.03	59±7.45
LDL(mg/dl)	47.16±6.24	61.16±18.31	43.83±13.78	61.33±20.47
VLDL(mg/dl)	12.05±0.95	15.56±1.40	20.35±5.34**	15.17±3.15
Triglycerides(mg/dl)	39.16±14.06	37.16±8.28	42.83±10.72	38.66±9.09

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 13:



28 Day Repeated Dose Oral Toxicity Study

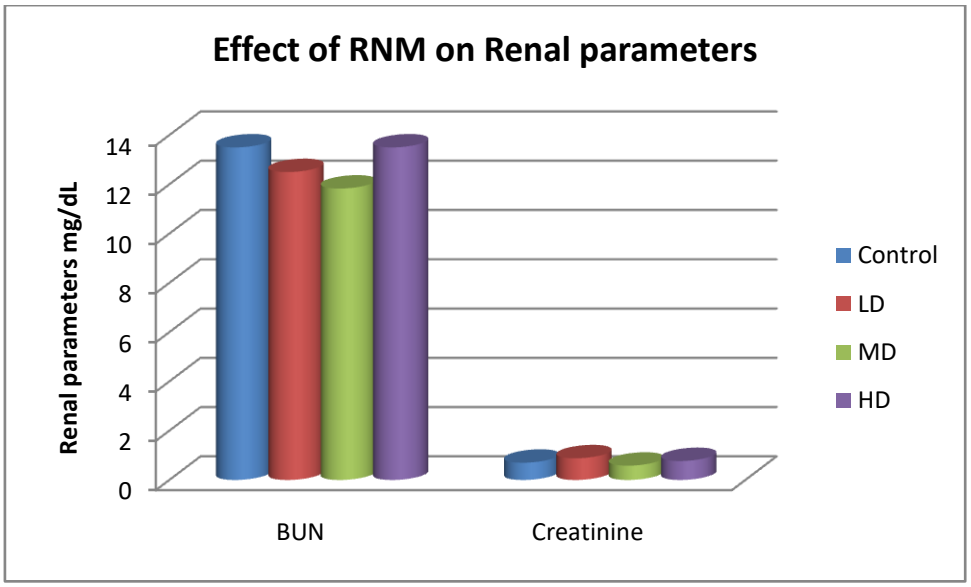
The results of the renal functions test conducted at the end of study, test groups revealed no significant changes in levels of renal parameters, when compared with control group.(Table 13)

Table13 effect of Rathi nagara rasa mezhugu on Renal parameters

DOSE(mg/kg)	control	LD	MD	HD
BUN(mg/dl)	13.5±1.87	12.5±3.39	11.83±1.94	13.5±2.88
creatinine(mg/dl)	0.7±0.08	0.88±0.17	0.58±0.17	0.77±0.24

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 14:



28 Day Repeated Dose Oral Toxicity Study

The results of the liver function test conducted at the end of the study, test groups revealed no significant changes in levels of liver parameters, when compared with control group.

Table14 Effect of Rathi nagara rasa mezhugu on Hepatic parameters

Dose (mg/kg)	Control	LD	MD	HD
Total bilirubin(mg/dl)	0.41±0.07	0.65±0.49	0.4±0.17	0.67±0.33
SGOT(U/L)	98.83±34.03	89±2.33	80.67±2.33	83.33±5.75
SGPT(U/L)	28.16±4.44	23.83±3.71	39.66±4.08	25.66±4.32

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 15:

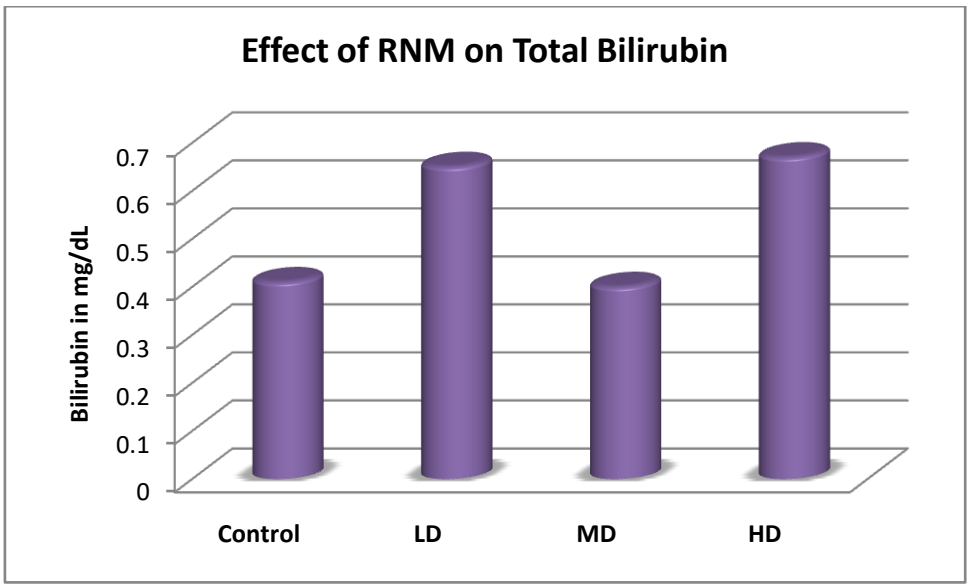
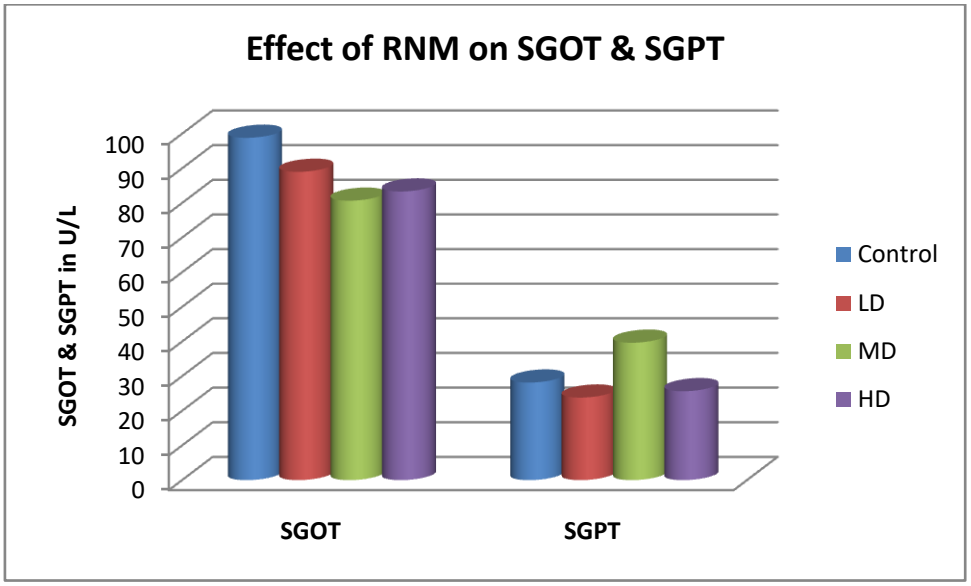


Figure 16:



28 Day Repeated Dose Oral Toxicity Study

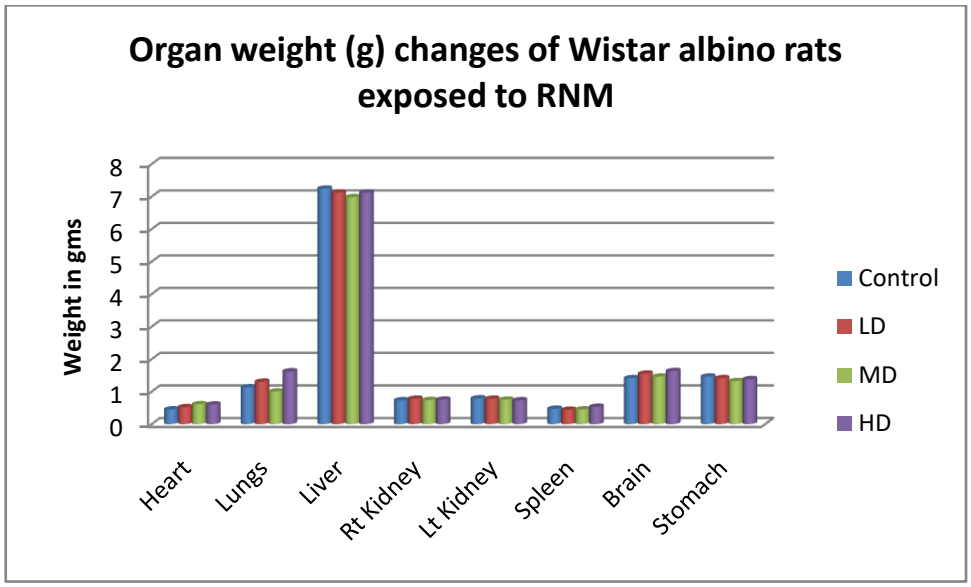
The Organ weight no difference in organ weight of control and test group observed after 28th days repeated oral toxicity study period, and satellite group was sacrificed after 14 days of drug withdrawal.(Table 15)

Table: 15 Organ Weight (g) Changes of Wister albino Rats Exposed to Rathi nagara rasa mezhugu

Organs	Control	LD	MD	HD
Heart	0.45±0.02	0.52±0.10	0.61±0.11	0.60±0.01
Lungs	1.13±0.21	1.3±0.34	1.4±0.02	1.62±0.51
Liver	7.24±1.77	7.12±1.12	6.98±0.11	7.12±1.65
Rt.kidney	0.73±0.07	0.78±0.41	0.74±0.43	0.75±0.20
Lt.kidney	0.79±0.09	0.78±0.07	0.75±0.01	0.73±0.19
Spleen	0.47±0.21	0.44±0.08	0.45±0.02	0.53±0.13
Brain	1.41±0.12	1.55±0.08	1.46±0.02	1.63±0.09
Stomach	1.26±0.16	1.41±0.09	1.32±0.16	1.38±0.18

Values were expressed as mean± S.D. for N=6 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 17:


Organ weight (g) changes of Wistar albino rats exposed to RNM
Weight in gms
8
7
6
5
4
3
2
1
0
Heart
Lungs
Liver
Rt Kidney
Lt Kidney
Spleen
Brain
Stomach
Control
LD
MD
HD

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF BRAIN

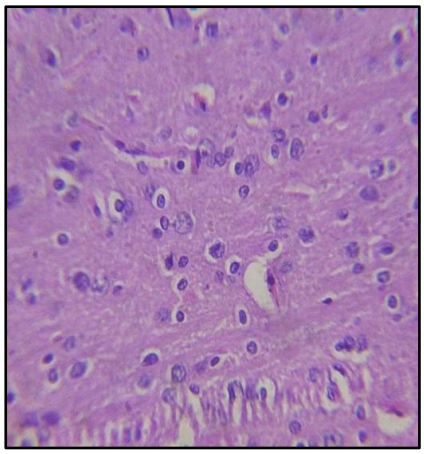


Plate A: Control Male

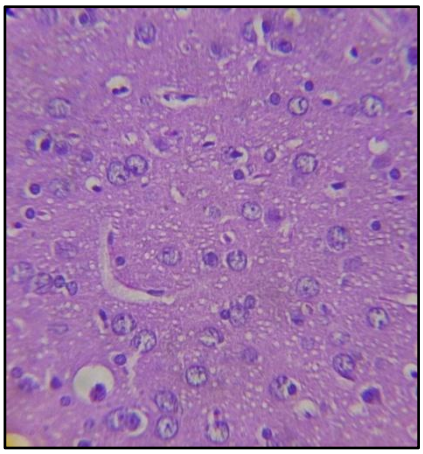


Plate B: Control Female

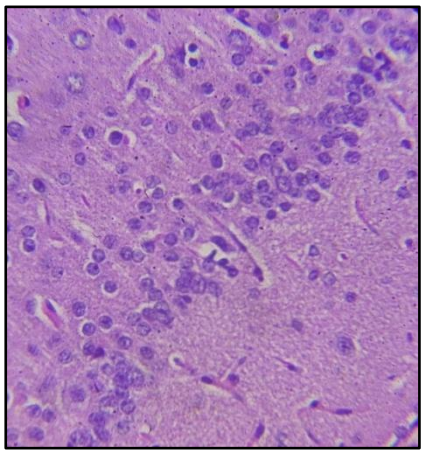


Plate C : High dose Male

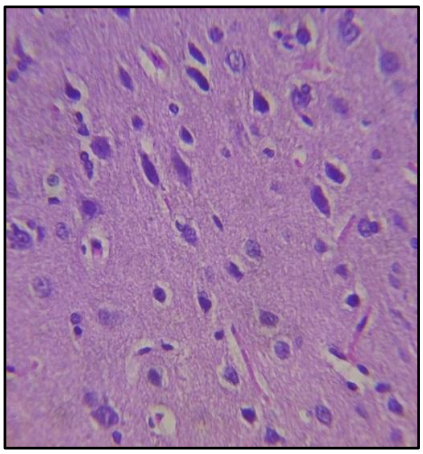


Plate D: High dose Female

Plate A: No signs of ischemic changes in the cerebral hemisphere of the brain sample

Plate B: Normal arrangement of neurons with proper inter neuronal space were observed in cerebrum

Plate C: Arrangement of neurons on cerebral cortex appears normal and dense. No signs of degeneration and other abnormalities

Plate D: The cerebral sections showed normal architecture in both cortex and medulla without any changes

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF HEART

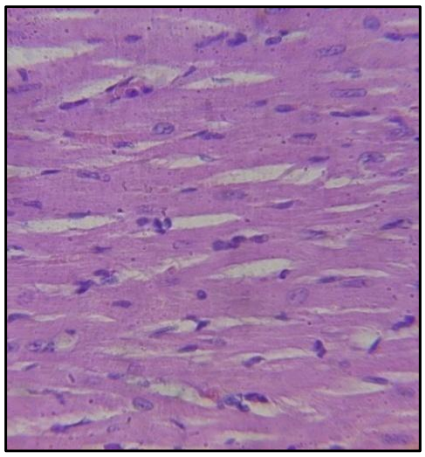


Plate A: Control Male

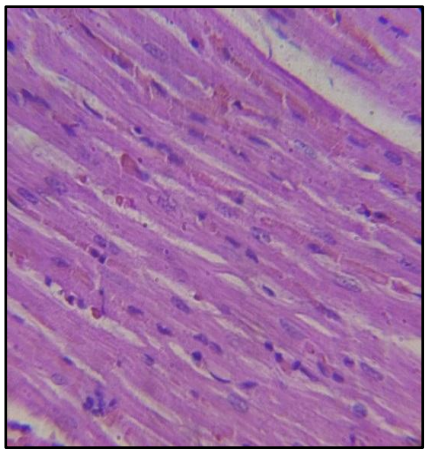


Plate B: Control Female

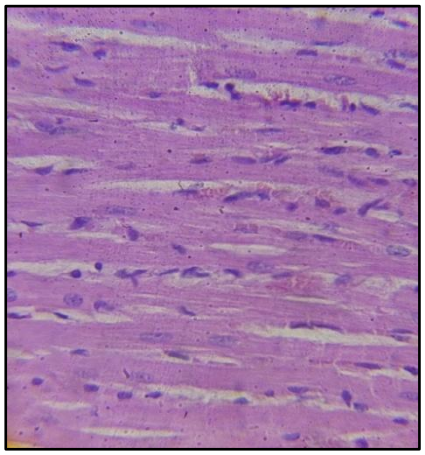


Plate C: High dose Male

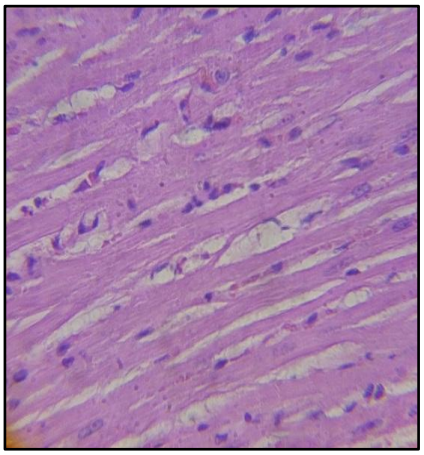


Plate D: High dose Female

Plate A: Sarcoplasmic region of myocardium appears normal

Plate B: Sarcoplasmic region of myocardium appears normal

Plate C: Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus.

Plate D: Fibres appears normal elongated and rod shaped

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF LUNGS

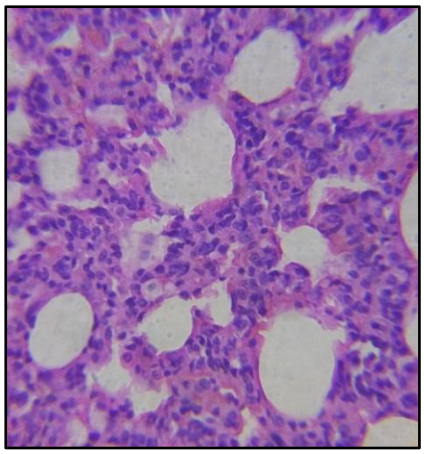


Plate A: Control Male

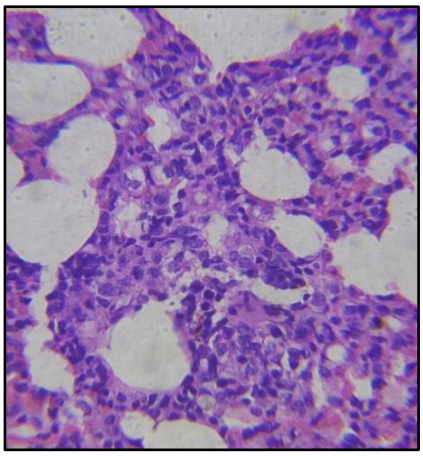


Plate B: Control Female

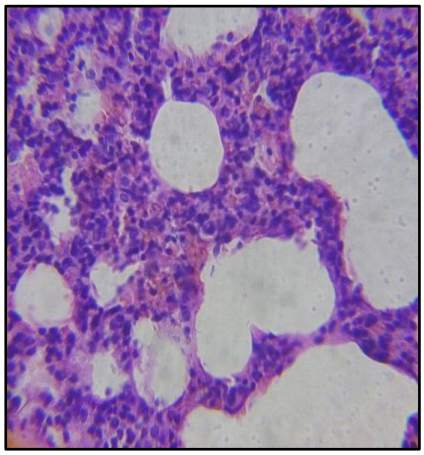


Plate C : High dose Male

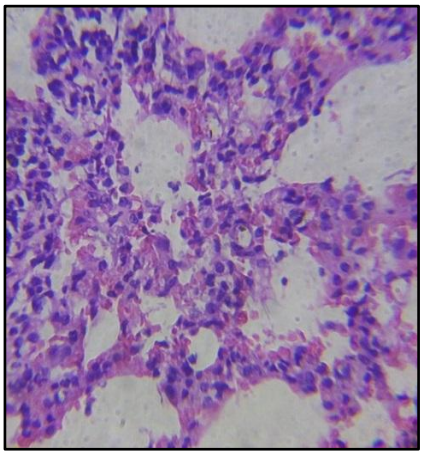


Plate D: High dose Female

Plate A: Arrangement of epithelial and muscular appears normal

Plate B: Lung parenchyma appears normal with regular arrangement of alveoli

Plate C: Bronchial blood vessels and connective tissue appears normal with no sings of pulmonary edema

Plate D: No signs of airway secretion and bronchial secretion

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF LIVER

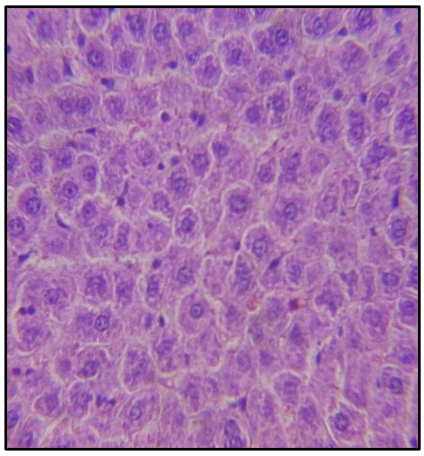


Plate A:Control Male

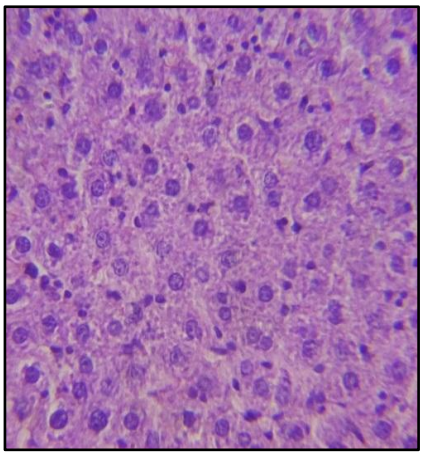


Plate B: Control Female

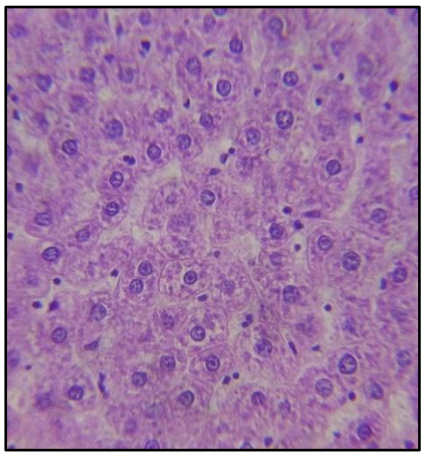


Plate C : High dose Male

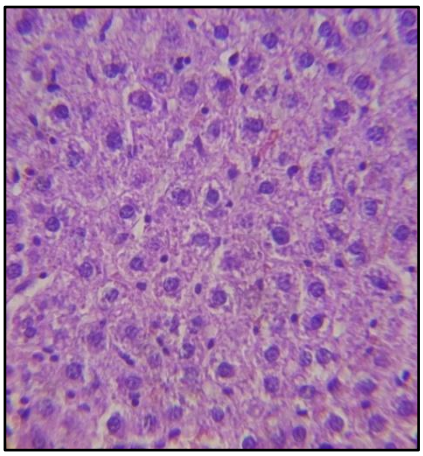


Plate D: High dose Female

Plate A: Section of liver showing normal, homogenous, intact hepatic parenchyma.

Plate B: liver showing normal hexagonal hepatic lobules with normal, regular radiated hepatic cords

Plate C: The centrilobular hepatocytes appears normal with stained cytoplasm.

Plate D: Liver parenchyma appears normal with no evidence of necrosis

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF STOMACH

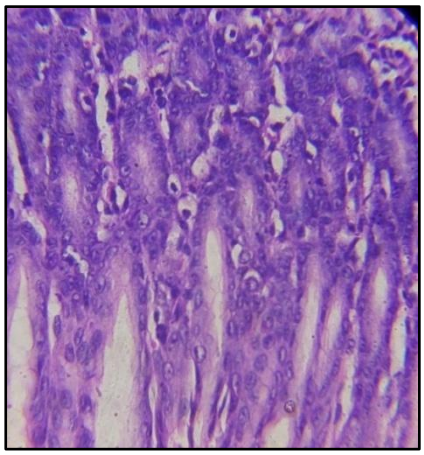


Plate A: Control Male

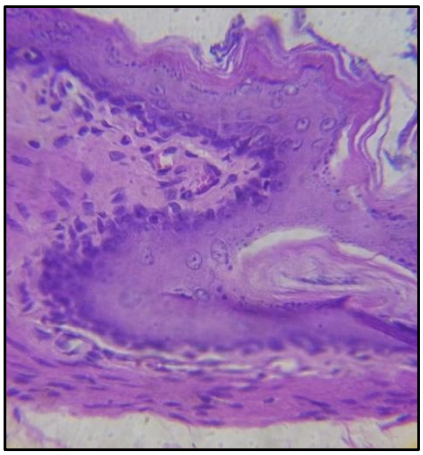


Plate B: Control Female

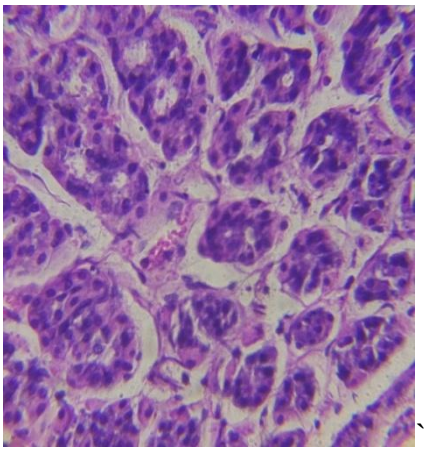


Plate C : High dose Male

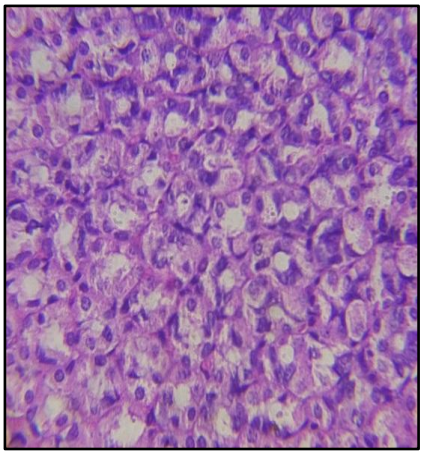


Plate D: High dose Female

Plate A: Appearance of Sub-mucosa and gastric glands appear normal

Plate B: No signs of ulcer and glandular degeneration were observed

Plate C: Gastric glands, gastric glands including secretary sheath appears normal.

Plate D: Mucosal wall appears normal with regular arrangement of connective tissue

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF KIDNEY

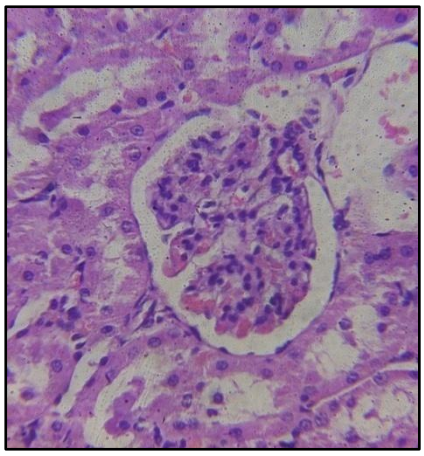


Plate A: Control Male

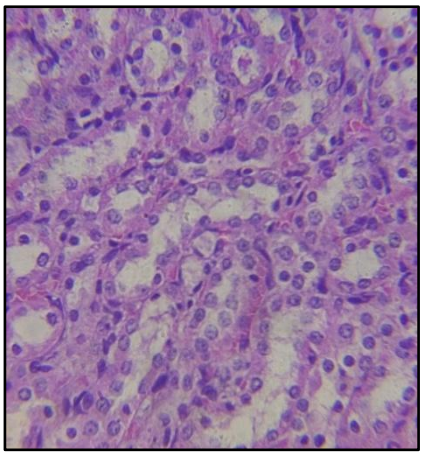


Plate B: Control Female

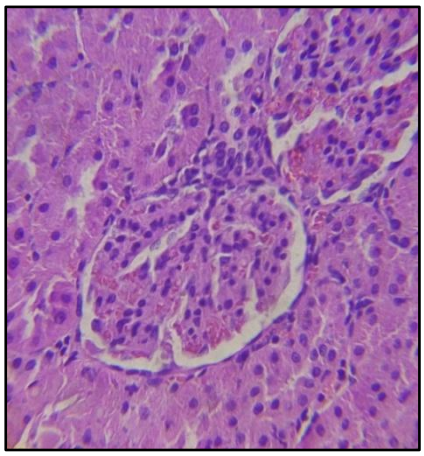


Plate C : High dose Male

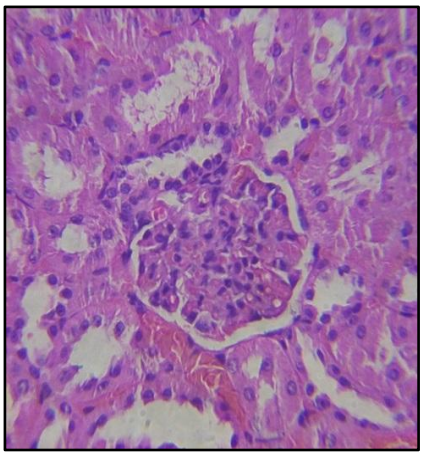


Plate D: High dose Female

Plate A: Glomerular Basement Membrane (GBM) separating between Capillary Space (Cs) and Urinary Space (Us) was normal.

Plate B: Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy

Plate C: Some renal tubules are hypertrophic, others are dilated.

Plate D: Basement membrane of the capillaries are thickened .Some renal tubules are hypertrophic

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF SPLEEN

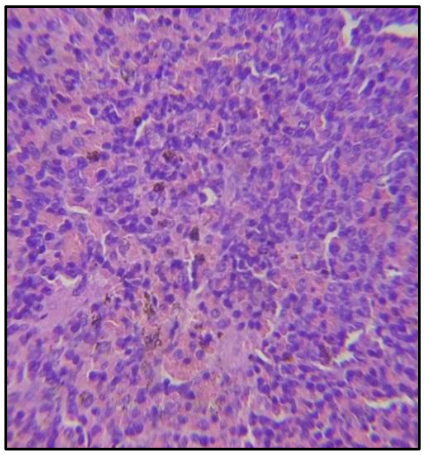


Plate A: Control Male

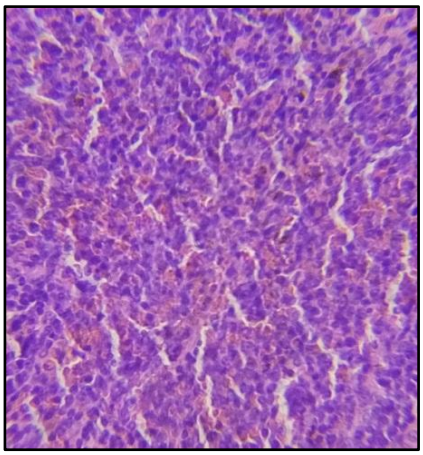


Plate B: Control Female

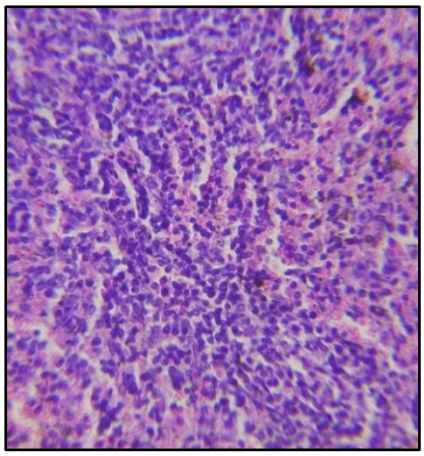


Plate C : High dose Male

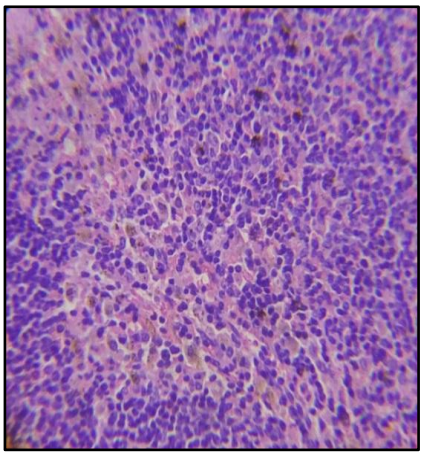


Plate D: High dose Female

Plate A: Appearance of splenic sinuses, Splenic cord and endothelial orientation was normal.

Plate B: Erythropoietic cells (EP) are scattered throughout the red pulp of both the samples. No abnormalities found in lymph node of both the samples

Plate C: Presence of marginal at the interface of the red pulp with the PALS and follicles was observed.

Plate D: Marginal vascular zone radiated in between red and white pulp

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF TESTES

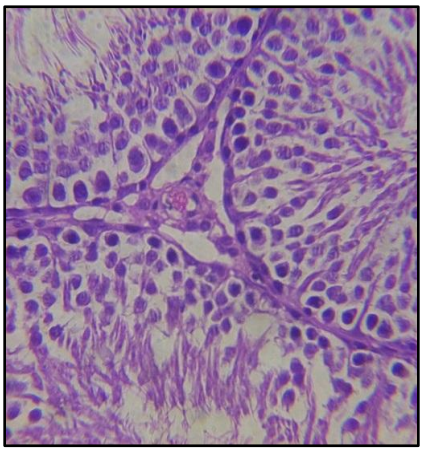


Plate A: Control Male

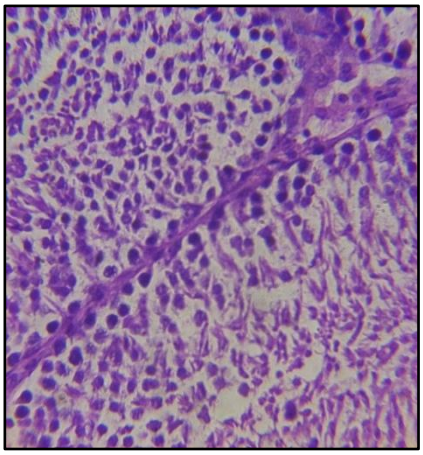


Plate B : High dose Male

Plate A: Normal sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus

Plate B: Sperm oriented towards the center of sertoli cells with cluster of tail projected outside was observed

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF UTERUS

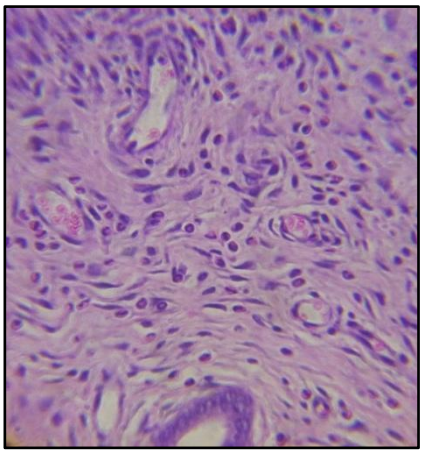


Plate A: Control Female

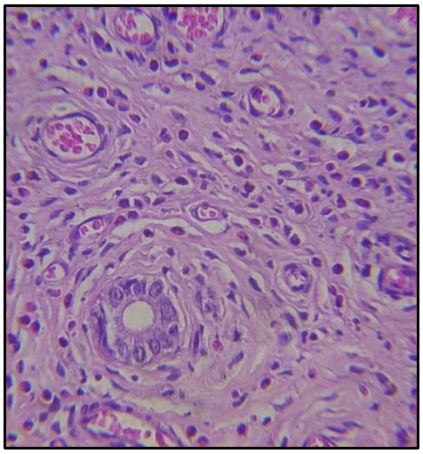


Plate B : High dose Female

Plate A: Appearance of endometrium, myometrium and uterine glands was normal

Plate B : Endometrial stroma; G, gland; M, myometrium; P, perimetrium; L, lumen exhibits normal histological aspect of endometrium and myometrium.

28 Day Repeated Dose Oral Toxicity Study

HISTOPATHOLGY OF UTERUS

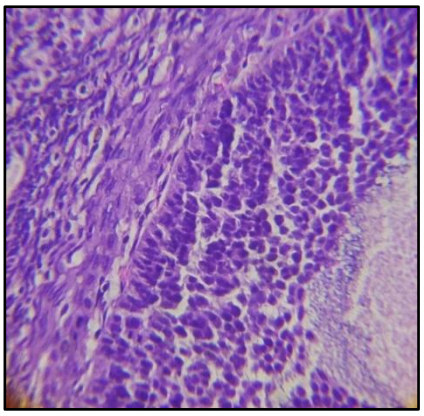


Plate A: Control Female

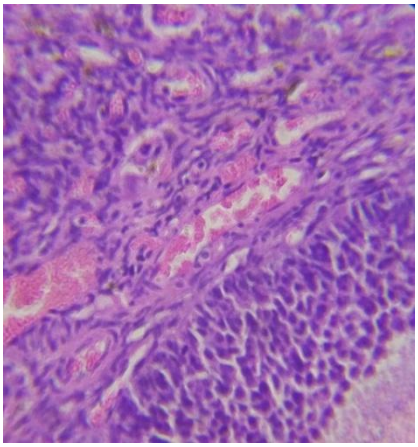


Plate B : High dose Female

Plate A: Follicular cells, cytoplasm and nucleus appears normal

Plate B : Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality

6. DISCUSSION

I have selected Rathi nagara rasa Mezhugu (RNM) to evaluate the safety profile. First the test drug going to the process of standardization for qualitative and quantitative analysis. The following analysis are:

- Physico - chemical analysis
- Bio-Chemical Analysis
- ICP-OES
- SEM Analysis
- The safety profile is evaluated by Acute and sub acute toxicity study on Wister Albino rats as OECD 423& 407 guideline.

The **Physico - chemical analysis** of RNM (Table: 1&2) concludes the following results

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Moisture is one of the major factors responsible for the determination of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The percentage of loss on drying of RNM was 1.98% (Normal range: 1-20%). Since the loss of drying of RNM is low, the stability of the drug is higher.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug. The total ash values of RNM were 2.09% (Normal range: 1-25%). Since the value of total ash in RNM is low, it implies that the inorganic constituents is Low this indicates the purity of the drug.

The Acid-Insoluble Ash limit Test is designed to measure the amount of ash Insoluble to diluted hydrochloric acid. Acid-insoluble ash value of RNM is 0.7% (Normal range: 0.1 – 10%) and it shows that a very small amount of the inorganic Constituent is insoluble in acid. It indicates the purity of the drug.

Extraction value determines the amount of active constituents in a given amount of the formulation when extracted with a solvent media such as water and alcohol. The water soluble and alcohol soluble extract values provide an indication of the extent of polar and non polar compounds respectively present in RNM. The extract values of Alcohol in RNM is 15.59% (Normal range:4-85%) and water is 3.77%(Normal range:485%). From the above result we conclude that water is a little better solvent of extraction than alcohol.

pH of the drug was 4.65 (Normal value:4-14). It denotes it is slightly acidic. Hence, in the oral administration of the drug it is expected to be absorbed quickly in the stomach. It reveals that RNM is expected to have better Bio-availability.

Bio Chemical Analysis of Rathi nagara rasa mezhugu indicated the presence of carbonate, calcium, magnesium, potassium, aluminum, iron, zinc, mercury, and alkaloid.

Phytochemical investigation to detect the presence of phyto constituents in formulation RNM reveals the presence of glycosides and fixed oil and fat.

The Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) of *Rathi nagara rasa* mezhugu results showed that the Heavy metals like Aluminum, Arsenic, Copper, Lead, Magnesium, and Nickel were found below detection level. Mercury (03.214 mg/L) was found within the permissible level in RNM. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like sodium, potassium, iron, zinc, Calcium, phosphorus (Table.5).

High Resonance Scanning Electron Microscopy (HR-SEM) analysis of *Rathi nagara rasa* mezhugu showed difference in size and reveals the particle size as 0.5 – 2 micron which is considerably less due to repeated calcinations and grinding. Hence the drug may have increased absorption. The particles in RNM had uneven distribution with agglomeration of particles. Agglomeration of the particles is due to repeated cycles of calcinations involved in preparation (Image.1).

Acute Toxicity Study was done as per OECD Guideline-423 with dose levels of 50, 2000 mg/kg b.wt. Throughout the 14 days of observation period, no mortality was observed in *Rathi nagara rasa mezhugu* treated groups. No abnormal behavioral signs were observed in treated groups. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. Since there was no mortality in dose of 2000mg/kg.wt, the LD50 cut off value falls on above 2000mg/kg b.wt. So the drug *Rathi nagara rasa mezhugu* comes under the Category-5 according to GHS (Globally Harmonized System for the Classification of chemicals) [60] as per the OECD guideline-423 and this provides direct relevance for protecting human and animal health.

Repeated Oral Toxicity Study was conducted for 28 days as per the OECD guideline-407. All animals from control and all the treated dose groups survived throughout the dosing period of 28 days. There was no mortality, morbidity and behavioral changes during cage side observation. Food and water consumption of the animals is significantly differed When RNM treated drugs compare to control group (Table7,8& Figure2,3). But they are within physiological limit, and this study reveals that it does not adversely affect the basic metabolic processes of the experimental animals.

On 29th day all animals are sacrificed and blood was collected. After blood collection, all the animals were euthanized for gross pathological examinations of all major internal organs. The blood samples were sent to a lab for hematological and biochemical analysis. The organs were weighed and preserved in 10% buffered formalin solution before sending for histopathological study. All the reports were statistically analyzed.

The results of hematological investigation, reveals no significant changes in all *Rathi nagara rasa mezhugu* treated groups as compared with the control groups except for total differential count, hematocrit but the values are within the normal physiological limit.

The results of biochemical investigations, in test groups there was significant changes present in VLDL Mid dose, when compared with the control group. But the values were normal biological limits.

The result of liver function test and renal function test, reveals no significant changes in all *RNM* treated groups as compared with the control groups.

Relative organ weights of treated animals when compared with respective control animals did not reveal any abnormalities..

The histopathological study, the vital organs such as brain, heart, liver, spleen, kidneys, lungs, testes and ovary were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Histopathology of vital organ revealed normal histological appearance when compared with the control.

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7. SUMMARY

Preclinical safety evaluation of ***Rathi nagara rasa mezhugu (RNM)*** was carried out for dissertation. The drug was chosen from the Siddha literature Anuboga vaidhaya navaneedham. The ingredients of Rathi nagara rasa mezhugu are metallic and herbal compounds like Rasam (Mercury), Ganthagam (Sulphur), Serankottai (Semecarpus anacardium). It cures the disease of Lingaputtru (penial cancer), Algulputtru (Cervix cancer), Araiyaappu (Adenitis), Kandamaalai (Cervical adenitis), Karunkuttam, Senkuttam (Leprosy), Megaranam (Syphilis), Kaalkai mudakku (Rheumatoid Arthritis). [6]

The raw drugs were procured from reputed raw drug shop, Broadway, Chennai and the drugs were identified and authenticated by dept. of Gunapadam, and by the Botanist, National Institute of Siddha, Chennai. The ingredients were purified and the medicine was prepared as mentioned in the Siddha literature. *Rathi nagara rasa mezhugu* was underwent physicochemical, phyto- chemical, biochemical analysis, heavy metal analysis by using ICP-OES, and particle size analysis by using HR-SEM. Acute and Repeated oral 28 day toxicity were conducted as per the OECD guidelines 423 & 407 respectively[58,59].

In *Rathi nagara rasa mezhugu*, the loss on drying at 105^{o}C was found to be 1.98%, it falls in between the limit range (1-20%). So the determination of moisture content shows the good stability of the drug test drug. The total ash in the test drug was found to be 2.09%, and the acid insoluble ash to be 0.7%. Both types of ash values were within the limits. The minimal level of *acid insoluble ash* shows the less extraneous matters that indicate the purity of the drug RNM.

The water soluble extract value of Rathi nagara rasa mezhugu is 3.77% and the Alcohol soluble extractive is 15.59%.As the test drug has more alcohol soluble constituents than water soluble, it would be non-polar. So the drug will have good bioavailability &intracellular distribution without possible accumulation inside the cells. The poor water solubility may prolong the duration of the drug action.

The pH of RNM was found to be 4.65 which means it is weekly acidic and safe for oral administration.

Bio Chemical Analysis of RNM indicated the presence of carbonate, calcium, magnesium, potassium, aluminum, iron, zinc, mercury, and alkaloid.

Phytochemical investigation to detect the presence of phyto constituents in formulation RNM reveals the presence of glycosides and fixed oil and fat.

The Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) of *RNM* results showed that the Heavy metals like Mercury (03.214 mg/L) was found within the permissible level in RNM. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like sodium, potassium, iron, zinc, Calcium, phosphorus. Some Heavy metals shows below detection level are Aluminum, Arsenic, Copper, Lead, Magnesium, and Nickel (Table.5).

HR-SEM analysis of *Rathi nagara rasa* mezhugu showed difference in size and reveals the particle size as 1.0 - 2.0 µ. The particles in RNM had even distribution with agglomeration of particles due to reported grinding. Hence the drug may have increased absorption.

In Acute Oral Toxicity study four different dose (50, 2000mg /kg.b.wt) of Rathi nagara rasa mezhugu were administered stepwise (step up) manner. Throughout the 14 days of observation period, no mortality and signs of toxicity were observed in RNM treated groups. The results of the Acute Oral Toxicity study indicate that the LD50 of RNM is more than 2000 mg/kg b.wt. So the drug RNM comes under the category 5 as per GHS (Globally Harmonized System of Classification).[60]

In the 28-days repeated dose oral toxicity study, there were no mortality and morbidity in all Rathi nagara rasa mezhugu treated groups.

Food and water consumption of the animals is significantly differed When RNM treated drugs compare to control group(Table7,8& Figure2,3). But they are within physiological limit, and this study reveals that it does not adversely affect the basic metabolic processes of the experimental animals.

Body weight of the animals is significantly differed When RNM treated drugs compare to control group (Table9,10 & Figure4,5). But they are within physiological limit.

The results of hematological investigation, reveals no significant changes in all *RNM* treated groups as compared with the control groups except for total differential count, hematocrit but the values are within the normal physiological limit.

The results of biochemical investigations, in test groups there was significant changes present in VLDL Mid dose, when compared with the control group. But the values were normal biological limits.

The result of liver function test and renal function test, reveals no significant changes in all *RNM* treated groups as compared with the control groups.

No abnormality was observed in histopathological examinations of all organs in RNM treated groups as compared with the control groups.

So the 'No Observed Adverse Effect Level' (NOAEL) of Rathi nagara rasa mezhugu may be greater than 468 mg/kg b.wt in Repeated Oral 28-day Toxicity study.

8. CONCLUSION

From the results of this analytical evaluation of the test drug Rathi nagara rasa mezhugu (RNM), it is inferred that quality and stability was good when prepared under the standard protocol mentioned in this study. Qualitative analysis of RNM reveals the Purity and Bioavailability of the drug As heavy metals were found to be within the permissible limit .so the drug is safe for oral consumption. The particulate size of the test drug was determined by SEM analysis. In vivo toxicity study reveals the drug RNM shows no mortality and signs of toxicity up to 2000 mg/kg.b.wt in acute oral administration. In Sub acute toxicity study there was significantly changes in hematological, biochemical parameter in RNM treated groups when compared to control group but the levels were within physiological limit. The histopathology report also confirms that there are no remarkable cellular changes at all the dose levels. And No Observed Adverse Effect Level (NOAEL) high dose level (468 mg/kg b.wt), which is ten times that of therapeutic dose.. Based on these results it can be conclude that, the dose level of Rathi nagara rasa mezhugu **3 to 4** kundri twice a day mentioned in the Siddha literature Anoboga vaithya navaneetham [6] is safe dosage for human consumption.

In future it is to be carried out to study the pharmacological activity and clinical trial to prove the efficacy of the drug.

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10. ANNEXURE

The following certificates are enclosed

1. Research Methodology and Biostatistics
2. IAEC Certificate for acute and sub acute toxicity study.
3. Authentication certificate for herbal plants.
4. Authentication certificate for metal drugs.

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~....M:..VEERASIVARAMAN.....

For participating as ~~Resource Person~~ / Delegate in the Twenty second Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 06th to 10th June 2016.

Dr.N.KABILAN, M.D.(S)
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.S.PUSHKALA,** M.D.,
REGISTRAR (FAC)

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

CERTIFICATE

This is certify that the project title.. Pre-Clinical Safety Evaluation of 'Rathinagara Rasamezhugu' - 49 Rats (20M+29F)

has been approved by the IAEC. Approval No: NIS/IAEC-II/14/2016

Prof. Dr. V. Baranalatchi
Name of Chairman/~~Member Secretary~~ IAEC:

Prof. Dr. K. Nachimuthu
Name of the CPCSEA nominee

~~Name of CPCSEA nominee:~~

V. Baranalatchi 29/08/16
Signature with date

29/8/2016

Chairman/~~Member Secretary~~ of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

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F.No:NIS/Gunapadam/Au/2017/8 14.07.17

AUTHENTIFICATION CERTIFICATE

Certified that the samples submitted for identification by **Dr. M. Veerasivaraman**, II year PG scholar, Dept. of Nanju noolum Maruthuva Neethi noolum, National Institute of Siddha, Chennai - 47, are identified as **Rasam- Mercury & Gandhagam-Sulphur** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

14/7/17

Dr. S. Visweswaran, M.D (s)
Head of Department
Department of Gunapadam
National Institute of Siddha
Tambaram Sanatorium, Chennai-47.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **Rathiranaga rasamezhugu** (Internal) taken up for Post Graduation Dissertation studies by **Dr.M.VEERASIVARAMAN** M.D.(S), II year, Department of Nanju Noolum Maruthuva Neethi Noolum, 2017, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Semecarpus anacardium Linn.f. (Anacardiaceae), Nut

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2942017

Date: 07-04-2017

Authorized Signatory

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